GROWTH AND DEVELOPMENT OF CHINOOK SALMON, *ONCORHYNCHUS TSHAWYTSCHA*: EFFECTS OF EXERCISE TRAINING, AND SEAWATER TRANSFER

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by

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To my loving and supportive parents, Craigie and Christina

PREFACE

It seems quite fitting that I write this short, ambling *preface* exactly one month after the *viva voce* examination. With every effort not to pre-empt the acknowledgements at the end of the thesis, I thought it necessary to write a wee jot of informal garb at the front. My thesis now has the necessary *viva voce* examination amendments, and a number of the other examiners' comments have received attention, specifically the MATERIALS AND METHODS sections of Chapters 3 to 7 (my apologies to those who read on and contest that they're still too wordy and repetitive of chapter 2!). A bit more '*oooomph*' has been added to Chapter 8 (*bit as yin saiz ower here, 'nae muckle!'*). I express gratitudes to both my external examiners for reading and criticising my thesis at short notice and in quick time. Furthermore, I acknowledge that this, the final product, has been enhanced by their efforts. Finally, I gratefully thank Professor Dominic Houlihan for permitting me the use of the computer facilities at the Zoology Department, University of Aberdeen, SCOTLAND, and thereby facilitating the 'doing-of' the neccessary corrections.

A great vote of thanks to one and all in New Zealand who ensured that my life '*downunder*' was a tremendously enjoyable and fun-filled experience. I have the photographs from '*Tandoori Palace*' and having studied them in great detail, I have decided that '*Kiwis*' are, on the whole, quite an ugly bunch!! I look forward to hearing from New Zealand again, and I hope that the correspondence will amount to more than just '*account overdrawn*' letters from the bank!

If I were to re-live my 'Land of the Silver Cloud' experience it would be little different, excepting that I would have ideally left your country after the **magnificent** 2nd All Blacks *versus* The British Lions, 1993 Rugby Test. I still haven't seen the game, but I heard the result whilst lounging beside a swimming pool in the heart of France, soaking up the sun, and awaiting the evening's nuptial festivities. It was a fine wedding, and I guess that big-big bro Nick will always have the excuse that he can't possibly be in wedlock as he (like the rest of his family and indeed the entire Scottish contingent) didn't understand a word of the actual marriage ceremony!

It was a pity that I had to leave New Zealand in such a rush due to the event mentioned above. I can't help feeling that I have unfinished business and celebrations to attend to, and so as a promise and a threat, and in the words of one who is considerably larger (and far wealthier) than myself, '*I'll be back!*'

Aye

Mike Dougan
16th July 1993

CONTENTS

SO LONG, AND THANKS FOR ALL THE FISH

(Douglas Adams, 1984)

ABSTRACT

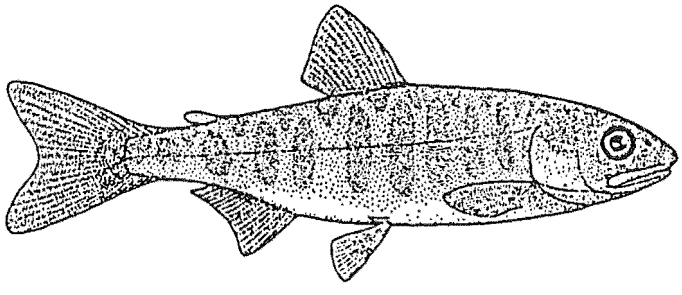
Low intensity (1-1.5 body lengths per second, $bl.s^{-1}$), short or long term exercise training significantly reduced the growth rate of underyearling (0+) chinook salmon, *Oncorhynchus tshawytscha*, at all stages of development from 'zip-up' fry to post-smolt (at 13-15 °C). Decreased growth with training was observed in both hatchery and wild chinook, and in 0+ sockeye salmon, *O. nerka*. Trained fish had lower weight specific (SGR) and linear growth rates (LGR), condition factor (CF), and exhibited lower gill Na^+-K^+-ATPase activity. SGR was greatest in pre-smolt fingerlings (*i.e.* fry), and then declined, whereas LGR was maximal during the period of parr-smolt transformation. Growth rate of chinook was accelerated in the warmer water of the laboratory (13-15 °C) compared to that experienced at a commercial hatchery (7-11 °C). It is evident therefore that exercise training does not appear to offer a viable commercial option for enhancing the growth of 0+ chinook, whereas 'warm water' rearing provided a measurable improvement of growth performance.

Hypo-osmoregulatory ability, assessed by 48 hour seawater challenge tests and gill Na^+-K^+-ATPase activity, of untrained chinook was determined over their first growing season, and was greatest during October-December. The smallest 'smolts' to survive in seawater were 2.5 g (65 mm). Fresh- and seawater growth rates were equivalent, provided the fish were transferred in a 'smolt condition'. Stunting and parr-reversion were rarely observed. Although plasma osmolality, and plasma concentrations of sodium and chloride were significantly greater in seawater resident fish, haematocrit was unaffected by salinity. Gill Na^+-K^+-ATPase activity increased two to four fold in seawater resident fish, and did not demonstrate the seasonal decline observed in post-smolts held in freshwater. After two to four months in seawater, visceral water content of seawater resident fish was higher than in freshwater reared fish of a similar size.

Swimming ability (*i.e.* critical swimming speed or U_{crit}, $cm.s^{-1}$) of chinook increased with fork length, and was unaffected by parr-smolt transformation. U_{crit} measurements of individual fish were repeatable. Low intensity exercise training did not enhance the swimming performance of chinook in subsequent U_{crit} tests. Whereas the swimming ability of 'wild' (*i.e.* naturally spawned) and hatchery reared, 0+ chinook did not differ, 0+ sockeye U_{crits} were significantly slower than those of 0+ chinook. U_{crit} tests tended to disrupt osmoregulation, causing decreased plasma chloride concentration in freshwater reared fish, and increased plasma chloride concentration in seawater resident fish.

CHAPTER ONE

GENERAL INTRODUCTION



CHAPTER ONE

GENERAL INTRODUCTION

SALMONIDS WORLDWIDE

The family Salmonidae is a biologically interesting group of freshwater fishes of the temperate and cold waters of the Northern Hemisphere (Tchernavin, 1939). The group comprises relatively few species, many of which are anadromous. Most salmonids are economically important natural resources. Adult salmonids - salmon, trout and charr - are commonly large fish with a highly prized, edible flesh (charr is spelt along the guidelines of Morton, 1980). As adults in particular, they tend to be aggressive and offer 'a good fight' to anglers. It is for these reasons, that there have been extensive attempts to establish several salmonid species in parts of the world outwith their natural range. The majority of these introductions have had limited success.

Historically, salmonids have been an exceedingly important food source for native peoples. More recently, they have been overfished both on- and offshore (Ricker, 1972; Netboy, 1974; Childerhose and Trim, 1980; Groot and Margolis, 1991). Although exploitation has been balanced to some extent by intensive culture of juveniles for the last 200 years or so, stocks worldwide are a fraction of those in the past. Nonetheless, spectacular, annual spawning runs may still be observed, and these are often a tourist attraction, particularly within National Parks.

SALMONIDS IN NEW ZEALAND

The family Salmonidae is phylogenetically close to the family Galaxiidae, sharing a common ancestor during the Mesozoic (McDowall, 1990b). However, unlike their cousins, none of the seven salmonid species resident within New Zealand waters are endemic. Introduction of salmonids make up a large proportion of the 19 teleost species that have been successfully released into wild habitats (McDowall, 1990c). Exotic transfers of salmonids were variously undertaken between 1860-1910, to improve sport and game fishing and to establish commercial fish canneries. Furthermore, the effect of introducing exotic fish species on the native fish fauna was largely overlooked, the indigenous species only being recognised as important in terms of 'trout food' (Thomson, 1922; Phillipps, 1924; McDowall, 1990b,c). Success of the individual salmonid species, in terms of watershed colonisation and subsequent distribution, varies quite considerably.

Atlantic salmon (*Salmo salar* Linnaeus, 1758), brook charr (*Salvelinus fontinalis* Mitchill, 1814), mackinaw (*Salvelinus namaycush* Walbaum, 1792), and sockeye salmon (*Oncorhynchus nerka* Walbaum, 1792) were introduced, and all maintain relatively small, 'wild' (*i.e.* naturally spawned), localised, naturally self-sustaining populations. Though all four species exist in waters of the South Island, only brook charr inhabit riverine and lacustrine environments of the

North Island. The wild populations of these fish complete their entire life-cycle in freshwater in this New Zealand. None of the species is actively sport fished or farmed to any great extent at present[1].

Brown trout (*Salmo trutta* Linnaeus, 1758) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) introductions have been tremendously successful with respect to their almost ubiquitous colonisation of the freshwater habitat in this country. Both species have established relatively large populations in most river systems throughout New Zealand (brown trout are found south of the Coromandel Peninsula, and rainbow trout, south of the Waikato River system)[2]. Although the vast majority of brown and rainbow trout ova were of the freshwater form of both species, there is good evidence that the anadromous forms (sea-trout and steelhead, respectively) were also introduced. Sea-trout (*Salmo trutta*) populations occur in many rivers (Scott, 1964; McDowall, 1990a,b), whereas steelhead are limited to rivers in the Hawke's Bay area (Lally, 1973a,b; McDowall, 1990b). Both species are targeted in many important freshwater tourist/sport fisheries, particularly in the North Island. Neither is farmed commercially as such, due to the 1983 Fisheries Act, which prohibits the sale of trout or trout flesh. However, both are stripped and reared during their early life for lake and river enhancement.

Chinook salmon (*Oncorhynchus tshawytscha* Walbaum, 1792) have established viable sea-running populations in many of the cool, east coast rivers of the South Island. Locally, chinook are called 'quinnat' - as the taxonomic name was *Salmo quinnat* at the time of introduction (Thomson, 1922). They have been a particularly successful species within New Zealand as most populations have maintained their anadromous life-history. Autumn runs of adult chinook are keenly fished by both surf-casters and up-river anglers. Recently the species has been somewhat overfished by inshore commercial trawlers (McDowall, 1990a; Unwin *et al.*, 1991) which has caused the spawning runs to decline. Chinook are commercially farmed throughout the South Island in seawater net-pens and freshwater ponds. Many other operators have set up ocean ranching ventures, although the viability of these is rather dependent on the number of salmon that are caught offshore by the commercial trawlers. There is currently renewed interest in expanding the 'natural' range of sea-running chinook in the South Island.

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1. Big Glory Seafoods Limited subjected Atlantic salmon 'smolts' to seawater transfer trials in sea-cages off Stewart Island in 1981 and 1982. The trials were not a success with most fish showing poor growth. Attempts to develop a broodstock also failed, and the trials were terminated after two years (Mr K O'Sullivan, personal communication, 1992). There is fresh interest in sockeye farming due to the valuable, scarlet flesh of this salmon. A sockeye broodstock has been cultivated in freshwater in this country for a number of years at a Government Hatchery; possibly the only part of the world where they are farmed in this way (McDowall, 1990b). The Regal Salmon Company are presently assessing the economic viability of on growing sockeye in sea cages off Stewart Island (Mr E Walker, personal communication, 1992).

2. Although both species do occur in areas north of these geographical limits, such populations are maintained by annual stocking of hatchery reared fry. Neither species has a truly viable spawning population north of the limits given in the text. The distributions of brown and rainbow trout therefore, reflect their naturally self-sustaining, wild populations.

HISTORY OF CHINOOK SALMON IN NEW ZEALAND

The sheer size of adult chinook (the largest salmon) makes them a valued and attractive fish for anglers. The first attempt to introduce chinook was made by the Hawke's Bay Acclimatisation Society from 1875-1879. Liberations of fish were widespread, from Waikato to Southland. Although there were a few reports of sea-run fish being caught and identified as chinook (Stokell, 1962), it is thought that by the end of the nineteenth century, there were effectively no chinook in New Zealand waters (McDowall, 1990b).

Lake Falconer Ayson, head of the New Zealand Marine Department, persuaded the Government of the day to fund another attempt to establish chinook. It was his firm belief that they could successfully be established here as a sea-running stock. A hatchery was built on the Hakataramea River - a tributary of the Waitaki River system - to cultivate the shipments. Official United States Fisheries Commission Reports (1901-1908), indicate that approximately two million ova were shipped to Hakataramea (roughly 500 000 fertilised ova per shipment in 1901, 1904, 1906, and 1907). The consignments were derived from anadromous 'fall' (autumn) run Californian stocks. The 1901 consignment was taken from Battle Creek spawners (US Fisheries Commission Reports, 1901-1902), with the other shipments taken from McCloud River chinook (Dr P Bontadelli, personal communication, 1991). McCloud River and Battle Creek are high country tributaries of the Sacramento River, California. The hatchery successfully reared 1.5 million young chinook, which were released (at various ages) into several stretches of the Waitaki. In 1905, adult chinook were caught returning from the sea, and one year later, spawning redds were observed in the Hakataramea, and in other Waitaki rivers thereafter. In May and June 1907, milt and 30 000 ova were taken from sea-run chinook, for hatchery rearing at Hakataramea (Thomson, 1922). Ova and milt were collected annually thereafter, until the hatchery was closed in 1942.

Populations of chinook were established in other South Island rivers due to 'straying' of adults and through stocking policies of the Marine Department and numerous Acclimatisation Societies. By 1921, adult salmon were readily caught along the east coast of the South Island from the Waiau to Clutha Rivers. This essentially remains the present day distribution despite concerted attempts to establish populations further north of the Waiau, and along the 'West Coast'. Whilst these efforts largely failed to produce sizeable sea-running populations, isolated chinook populations do exist in a few West Coast rivers. Limited evidence also exists that suggests chinook may have successfully established small, sea running populations in some North Island rivers (Watson, 1980; Hicks and Watson, 1983; McDowall, 1990a,b).

The largest salmon runs are found in the cool rivers of Canterbury; the Waimakariri, Rakaia, Rangitata, and Waitaki Rivers. Though the annual runs in these rivers are relatively large, they are smaller than those in comparable North American rivers, due to the low annual recruitment. The Canterbury rivers are broad, braided and unstable (greywacke gravel), offering an inhospitable rearing environment, particularly in the main river stem. Although the tributaries are often small, stable, single braid, spring fed streams, they are susceptible to flash

floods from rapid snow melt, cloud bursts, and heavy 'nor-west' rain during the winter and spring.

Despite the expenditure of considerable time, energy and money to establish chinook, the status of the species is in decline, due to a number of factors. High country spawning streams have been lost to chinook with the advent of hydroelectric dam construction across many rivers. Intensive farming and water abstraction practices cause intermittent reductions of both river water quality and quantity; frequently during critical periods of the salmon life cycle. Overfishing in the marine phase is another cause of population decline (McDowall, 1990a; Todd and Unwin, 1990; Unwin *et al.*, 1991).

The news is not all bad. Aquaculture of chinook by various means, is on the increase. The success of ocean ranching ventures may depend on the size of the coastal trawler fishery. Nevertheless, seawater net-pen, and freshwater pond rearing are important developments, producing relatively large fish of high quality (in 1990, there were two facilities licensed to farm chinook in freshwater, and a further 22 seawater, net-pen licences: McDowall, 1990b).

HISTORY OF SOCKEYE SALMON IN NEW ZEALAND

Sockeye salmon were successfully introduced from a single consignment of 500 000 Fraser River ova in 1902 (an earlier shipment, shipped in 1900, died *en route* to New Zealand). At the time of their liberation, millions of North American sockeye were harvested annually by canning companies, and it was hoped that a large anadromous population of sockeye would establish; enabling the development of such an industry here (Hardy, 1983). However, after stocking in the Waitaki River system, there was only limited evidence of sea-run fish (Thomson, 1922; Stokell, 1955; and Scott, 1984). Instead, sockeye produced self-sustaining 'landlocked' or 'lake-dwelling' populations.

Confusion exists as to the exact nature of the initial stock. In their natural range, sockeye exist as three separate forms, sockeye (anadromous); kokanee (non-anadromous, lake dwelling); and residual (lake-maturing progeny of anadromous sockeye). All three forms are self-sustaining and are distinguishable (Ricker, 1940). Hardy (1983) queried whether the sockeye sent to New Zealand may have been derived from the kokanee form. The 1902 shipment came from the 1901 collection of ova from Lake Shuswap, on the Fraser River (Hardy, 1983). Lake Shuswap carries both sea-running sockeye, and lake-dwelling kokanee populations. Hardy (1983) speculated that perhaps by mischance, eggs collected for transport to New Zealand were of kokanee, rather than sockeye origin. However, New Zealand sockeye do not assume the crimson and olive coloration, strikingly obvious in the North American kokanee (and sockeye) spawners. The grey-green spawning coloration of the New Zealand sockeye is similar to that of the residuals.

Scott (1984) and McDowall (1990b) argue that initially, there may well have been many juveniles that migrated to sea. However these would in the main have become disoriented, failing to navigate back to their natal rivers. Such failing would strongly select against seaward

migrating tendencies in the population, and thereby the population would become increasingly freshwater resident. Ricker (1938, 1940, 1959, 1972) states that sockeye naturally have strong proclivity for freshwater residence, especially when downstream barriers to migration, or rapid dispersal from river mouths exist. Therefore, it would appear that despite being descended from anadromous stocks, the New Zealand sockeye quickly assumed a freshwater existence.

Franklin (1989) successfully transferred New Zealand sockeye to seawater as yearling smolts. These fish were derived from sockeye that had been resident wholly within freshwater for the previous 80-90 years (Franklin *et al.*, 1992). Work done in the Northern Hemisphere indicates that all three forms of sockeye will successfully transfer to, and grow in seawater (Burgner, 1991). Scott (1984) reasoned that sockeye in New Zealand cannot be correctly described as sockeye (lack of anadromy), kokanee (spawning coloration) or residuals (lake-maturing progeny of an anadromous stock). He proffers the term 'non-anadromous', and McDowall (1990b), 'lake-dwelling', sockeye.

It is an impressive achievement that sockeye were established (albeit in freshwater) in New Zealand from a single consignment of 500 000 ova, of which only 160 000 fry were viable (McDowall, 1990b). That no more introductions, and attempts to produce an anadromous population eventuated, is probably due to the successes achieved with the introduction of chinook. New Zealand sockeye presently have a restricted distribution, and as with chinook, are less abundant than they were. At the time of their introduction, sockeye were released into many stretches of the Waitaki River system. However, the construction of hydroelectric dams has limited the areas available to sockeye. The present wild stock is largely restricted to Lake Ohau. The Lake Benmore population is all but extinct in the wild, with the building of the Ruataniwha Dam, preventing the upstream migration of spawners (McDowall, 1990b). Spawning occurs almost exclusively in Larch Stream, a cool, spring fed tributary of Lake Ohau (Graynoth *et al.*, 1986).

LIFE HISTORIES

The 'classical' life history of salmon related by Childerhose and Trim (1980) and others, relate the conquering of the hazardous journey of life, by a masterful fish. Whilst these reports are largely accurate, they are based as much on fiction and anecdote, as they are on fact. Though each salmonid species has a unique life history strategy, aspects of each are common to the rest. The natural history of chinook and sockeye are quite different, and there is considerable variation within each species.

Within North America, chinook exist as two 'races'. Gilbert (1913) was the first to document the different races in terms of the age that the fish migrate to the sea. He termed fish that migrate within their first year, sea-type (now called ocean-type), stream-type referring to those that remained in freshwater for at least a year. The age at seawater entry was determined by back calculation, using scale analysis. Rich (1920) noted differences in the timing of the spawning runs, ocean-type tending to run in the autumn; stream-type in the spring. Ocean-type

chinook tended to dominate runs from 40° to 56°N; stream-type more common north of 56°N (Healey, 1983). Where the two races overlap, stream-type tend to predominate in high-country tributaries, and ocean-type in the more coastal streams. Life in the marine phase also differs between the races. Ocean-type are coastal, remaining within inshore waters, whereas stream-type migrate offshore during their first year in seawater, and undertake more extensive migrations (Healey, 1980a,b; Hartt, 1980). According to the literature, and the New Zealand chinook are descended from fall run, ocean-type, Sacramento River chinook (United States Bureau of Fisheries Reports, 1899-1909).

Sockeye, as mentioned above, have three recognisable forms; the anadromous sockeye; the non-anadromous kokanee; and the non-anadromous offspring of anadromous sockeye, the residuals. The New Zealand sockeye are descended from anadromous, fall run, Fraser River sockeye.

Chinook salmon

Chinook salmon have received extensive study for many years, both in New Zealand and overseas (Rutter, 1904; Gilbert, 1913; Rich, 1920; Hobbs, 1937; Parrot, 1971; Finlay, 1972). More recent studies have also been undertaken (Flain, 1981b; Field-Dodgson and Galloway, 1985; Unwin, 1986; Field-Dodgson, 1988; Davis and Unwin, 1989). Although the account below is based on wild fish of the Rakaia River, particularly those entering Glenariffe Stream, it probably applies to most other chinook runs in New Zealand.

Maturing adults migrate upstream from the sea from early November to late May, with the peaks at the river mouth in early March, and on the high country spawning grounds, toward the end of April. Spawning occurs in moderately swift flowing water over coarse gravel and cobble river bed. Females locate suitable gravel, frequently at the tail of a pool, and excavate small depressions (egg pockets) with flicks of their tails. Most often a single male approaches, and the pair discharge eggs and milt. Fertilised eggs (eight millimetres in diameter) sink into the egg pocket, and are then covered with gravel, as the female excavates another egg pocket immediately upstream. Spawning bouts continue in this way, until the female is 'spent', and the final egg pocket is covered. The entire nest is termed a redd, and it may take several days to complete. The average size of a chinook redd is 15.4 m^2 (Field-Dodgson, 1985). The female stays over the redd, protecting her progeny for as long as she is able to hold position, the male either swims off in search of other spawning partners, or is swept downstream, exhausted. Eventually, all the adults die, rotting in the streams and rivers. Stokell (1955) colourfully described the appearance of chinook spawners in New Zealand as '*A spawned quinnat with its colour dulled, its fins worn, its body mutilated and commencing to putrefy is a pitiable sight. Death does not come to them immediately after spawning but after a few days feeble struggling with the current.*'

Development of the embryo is dependent on temperature (Beacham and Murray, 1989, 1990), taking longer at cooler temperatures (15 °C or greater is lethal to salmon eggs; Fry, 1979). Alevins hatch out of the eggs after 4 to 6 weeks, and stay within the security of the redd

until they have depleted their external yolk sac, and have almost completely sealed their abdomen ('zip-up' fry). Feeding excursions may occur prior to leaving the redd for good (Field-Dodgson, 1988). Most fry (32-35 mm fork length, 220-270 mg wet weight) emerge from redds during the night, from August to early November. Peak emergence is in mid-September (Unwin, 1981). As much as 90-98% of the annual production of fry outmigrate to the river main stem within a few hours of emergence due to intraspecific competition, territoriality (Hopkins, 1981; Unwin, 1986), and poor swimming ability (Thomas *et al.*, 1969). Annual production has been estimated (from trapping data) to range from 275 000 to 3 752 000 fry (Unwin, 1986). Production is dependent on the number of spawners and the number and severity of any flooding events.

The fry that remain in the tributaries are strongly rheotactic, defending territories, and feeding on a catholic diet of adult and larval insects (*Deleatidium* spp., chironomids, other dipterans, and tricopterans; Sagar and Glova, 1987). Chinook parr are strikingly coloured with a greenish olive back, greenish yellow sides and a grey-white underbelly. There are small, grey-black dots along the back and larger grey blotches that extend laterally, and are interspaced with large oval marks along the sides (the so called 'parr marks', *see* CHAPTER 2, Figure 2.1). The fins of parr are yellow, with white leading edges. After 2 to 3 months feeding, most of the remaining chinook (90 000 fish, 60-90 mm fork length, 2-9 g wet weight) migrate down river as underyearling smolts (Unwin, 1981, 1986). Externally, the fish has changed; the back is a deeper green and the sides are uniformly silver (parr marks are 'lost' as purine crystals are laid down in the dermis). The coloration of the fins has faded to a paler light grey[3]. The physiology and behaviour of the fish have also changed (Schreck, 1982a). Smolts are freshwater fish that are 'pre-adapted' to life in seawater. They lose territoriality, form shoals, and migrate downstream during the hours of darkness. The changes associated with smolt development are collectively termed parr-smolt transformation (section below).

Some ocean-type chinook do not migrate to sea until they are a year old. The life of these fish is uncertain, but from work done in the Northern Hemisphere, the majority of males spawn precociously (Healey, 1991). Salmonids commonly show precocity, the term being defined as sexual maturation whilst in a juvenile form. The abundance of precocious males on the spawning grounds is unknown. Chinook adults die soon after spawning, and whilst this would also seem to be the fate of most precocious males, some may recover from spawning, and migrate to sea the following spring (Robertson, 1957). However, as females predominate in the yearling population of smolts on their seaward migration, the majority of precocious males probably do die post spawning (Leyzerovich, 1973; Mitans, 1973; Dalley *et al.*, 1983; Myers, 1984).

In New Zealand, spawning grounds are rarely more than 200 km from the river mouths;

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3. Parr is a term that is traditionally used to describe a certain stage of development in juvenile Atlantic salmon. Pacific salmon at the same stage of growth are normally given the more descriptive term; fingerling. Smolt, on the other hand, is in common usage worldwide. Smolt refers to fish that are able to osmoregulate in seawater, or fish that are on their downstream migration (Netboy, 1974).

this distance is navigated by the smolts within a few days. None of the main South Island salmon rivers has large, stable estuaries, in contrast to the natural chinook rivers of North America (Reimers, 1973; Healey, 1980a,b, 1982, 1991). Residence time within estuaries is therefore of short duration compared to that of North American chinook (Davis *et al.*, 1983; Healey, 1982; Kjelson *et al.*, 1982). In the estuarine environment, chinook feed on a range of prey, largely depending on the estuary (Eldon and Greager, 1983; Eldon and Kelly, 1985).

The life of chinook in the marine phase is almost totally unknown in New Zealand. However, fish of 300 mm and greater have been caught off Banks Peninsula. Fish have been taken on red cod (*Physiculus bachus*) and barracouta (*Thyrsites atun*) feeding grounds (at depths of 50-60 m). Additionally, salmon are found in many natural east coast harbours of the South Island; Otago, Timaru, Akaroa and Lyttleton. Trawlers have caught salmon at sea between 41° and 48°S, and up to 48 km offshore[4]. Samples of salmon caught at sea were found to have taken a krill-like crustacean (*Munida gregaria*), pilchard (*Sardinops neopilchardus*), and other fish species (Flain, 1981a). Salmon flesh has been observed in the stomachs of kahawai (*Arripis trutta*), southern kingfish (*Rexea solandri*), and school shark (*Galeorhinus australis*). There have been recent moves by the Ministry of Agriculture and Fisheries (MAF, currently entitled the National Institute of Water and Atmosphere, or NIWA) to obtain more data on the biology of chinook at sea, in particular their distribution, diet and marine migrations. This work has shown that chinook of all sizes preferentially feed on Euphausids, especially *Munida*. When *Munida* are scarce, stout sprat (*Sprattus muelleri*) and then hoki (*Macruronus novaezelandiae*) were the next most common prey items taken (Mr JRE Sykes, personal communication, 1992).

Most somatic growth occurs at sea, the 70 mm smolts returning as two year old's at 580 mm, 2.3 kg; three years 760 mm, 5.0 kg; four years, 960 mm, 6.8 kg. Fish older than four are extremely uncommon in New Zealand (Flain, 1972a,b). Size at return is variable depending upon brood year, and growing conditions at sea. In addition, the size of the runs varies annually, and is possibly cyclical (McDowall, 1990b). Although the age structure of the spawning population fluctuates, the dominant age class has always been three years old (3+), followed by four years (4+), and then two years (2+; Finlay, 1972; Flain, 1972b). This pattern is in contrast to the ancestral Californian stocks, where the dominant age class was four year old fish (Clark, 1929)[5]. North American stocks have fish up to seven years old (Scott and Crossman, 1973), although these are increasingly uncommon (Healey, 1991). Presently, two year old fish (99% of which are males) comprise 20-25% of Californian chinook spawners (Dr FW Fisher, personal communication, 1992); a similar situation is found in this country - two year old's may account for up to 30% of spawners. Average age at spawning in Californian

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4. Healey (1986b, 1991), detailing North American stocks, has shown that ocean-type, fall run chinook remain in coastal, inshore areas throughout their marine life: less than 50 km from the shore and at depths of 40-75 m. Stream-type, spring run chinook, however, undertake more extensive offshore migrations.

5. New Zealand chinook in general may be considered as precocious, as on average, the age at spawning is one year less than in North America. However, size at any age is equivalent (Finlay, 1972).

chinook has decreased since the days of Clark (1929) due to hatchery practices and commercial fishing pressures (Ricker, 1981; Healey 1991; Drs T Mills and FW Fisher, personal communications, 1992).

It is not known what spurs the fish to begin its homeward migration, but increasing blood titres of sex hormones, and rate of growth are important. Another largely unknown facet of salmon life-history, is the means by which salmon are able to navigate from open ocean to natal stream. Taylor (1986) produced evidence that chinook can orientate within magnetic fields. Quinn (1982, 1984a; Quinn and Leggett, 1987) has published a number of papers proposing models that salmon are able to use the earth's magnetic field 'as a map' to navigate through the ocean. Homing to the coastline therefore, may be directed by processing geomagnetic information. Haslar (1971) has shown that homing in freshwater may be controlled by dissolved 'odours'. Nordeng (1977) proposed a 'pheromone' theory, and Quinn and co-workers (1983) presented evidence in favour of the importance of pheromones. McIsaac and Quinn (1988) have demonstrated that riverine homing may have an innate, hereditary component.

Homing in salmon is remarkably accurate, most adults returning to the stream of their birth. However, Quinn (1984b) raises the evolutionary value of straying, the corollary of homing. In his hypothesis, straying would be most evident in populations of fish that spawned in unstable river systems, or in areas where there was a high degree of similarity between rivers. Both these factors could be attributed to the east coast rivers of the South Island. Additionally, chinook from Californian stocks (Sacramento and Klamath Rivers) have been recorded as having 10-13% straying rates (Snyder, 1931; Sholes and Hallock, 1979). McIsaac and Quinn (1988) have recorded straying in several other chinook salmon stocks. Straying in New Zealand chinook therefore may have been an important element in the establishment of stocks, and in their continued survival.

Up-river migration of salmon is influenced by a number of factors. Although the main run is during the autumn, individuals leave the sea at all times of the year. During the autumn, a southerly change, or a 'spring' tide spurs the fish to swim from the wash into the 'gut' of the river. Lunar cycle, coloured water after heavy 'nor-west' rain, and elevated river flow encourage salmon to run (Petrie, *in preparation*; Mr S Petrie, personal communication, 1992). However, as the autumn progresses, and the urge to spawn intensifies, fish will swim up river in all water states, and at all times of the day.

Sockeye salmon

The life history of the New Zealand sockeye salmon is much less complicated than the chinook, due to its lake dwelling habit. Whilst sockeye have never had an extensive distribution, established solely in the Waitaki River system, they are presently limited in the wild, to Lake Ohau, spawning in one of its tributaries, Larch Stream. The general biology of the sockeye salmon is much the same as the chinook. Therefore, only the major differences will be detailed below.

Maturing sockeye migrate from Lake Ohau during early autumn (March) to the cool,

spring fed water of Larch Stream. The migration lasts only a couple of weeks. The age structure of the spawning population varies annually, with adults aged two to four years, although some fish do not mature until five years old. Virtually all spawning is carried out within Larch Stream, in finer gravels, and smaller redds than those built by the chinook (probably due to the smaller size of the adult fish). Spawning occurs during a two to three week period in late March (Graynoth *et al.*, 1986; Graynoth, 1987).

Embryological development is quite rapid in Larch Stream. The early life history of sockeye alevins is much the same as for chinook. Emerging fry migrate from Larch Stream, almost immediately after leaving the redd, to Lake Ohau. As fry, sockeye have short, elliptical parr marks that extend slightly below the lateral line. Dark spots are visible along the bluish green back. Upon transition to the lake, the parr marks become less distinct, with the sides developing a more silvery appearance, and the hue of the back darkens.

Sockeye fry are pelagic predators, and have a varied diet of planktonic animals. However, growth of juvenile sockeye is slow due to the paucity of prey in Lake Ohau. New Zealand lakes tend to be biologically poor in terms of productivity, and as a result, planktonic animals are sparse (Graynoth *et al.*, 1986). As the fish grow, larger planktonic species, aquatic insects and native fish (common bullies, *Gobiomorphus cotidianus*) are found in the diet. Growth of sockeye, at any age, was greater in Lake Benmore than in Lake Ohau (Graynoth, 1987). However, with the impoundment of Lake Ohau, the Benmore sockeye have become extinct in the wild, although their descendants continue as the broodstock sockeye population at a Government Hatchery (McDowall, 1990b).

LIFE HISTORY OF PACIFIST SCOTSMON

Scotsmon are born with their hands on their very small tools. This peculiar habit persists throughout development and is made possible at all times by the wearing of short skirts. At maturation an allometric pattern of growth occurs. The very small sexual organs waste away, becoming vestigial, as energy is directed to the rapid growth of the mouth parts. Development of the arm musculature is also observed at this time, but it is widely believed that this is not a genotypic character, but rather a phenotypic one, brought on by the rapid (and continual) stroking and fondling of the (by now very, very) small sexual organ. Skirt wearing persists during adulthood, even when the individual is transferred to a habitat where men are men. Reproduction is thought to be achieved asexually, but this has yet to be confirmed. It seems unlikely, due to the microscopic nature of the genitals, that sexual reproduction is possible. Parturition has never been observed in the wild, but many recordings have been made of unusual sounds coming from the 5th floor toilets. Often after these brief and odorous episodes the Scotsmon can be heard crowing loudly to other individuals, presumably to inform the group of the safe arrival of another member. On a number of occasions newborn infants have been found blocking drains within the habitat. The average life expectancy of Scotsmon is far too long and the population should be controlled more effectively, than has been the case in the past *(courtesy of Glen William Davidson, 1992).*

PARR-SMOLT TRANSFORMATION

Parr-smolt transformation, or 'smoltification', is a developmental period of some salmonids that modifies the physiology and behaviour of the animal from a freshwater adapted fish, to a seawater adapted fish. The importance of parr-smolt transformation in salmonid life history, is apparent from the number of papers on this topic in the scientific literature. The increase in publications mirrors the growth of the salmon farming industry. There have been several reviews (Hoar, 1976, 1988; Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980; Langdon, 1985; Barron, 1986; McCormick and Saunders, 1987), at least three international conferences exclusively on the topic (Bern and Mahnken, 1982; Thorpe *et al.*, 1985; Hansen *et al.*, 1989a), and others which have partly addressed the problem (Dadswell *et al.*, 1987).

Whilst this plethora of information exists, the exact nature and regulation of the parr-smolt transformation is unknown. Many factors involved in the process have been determined, but control of the entire mechanism is uncertain. The whole process is somehow under an endogenous circannual rhythm, first demonstrated by Eriksson and Lundqvist (1982), and occurs during predictable seasons (spring, early summer) under normal conditions. If fish do not enter the sea, they 'desmoltify', *i.e.* readapt to the freshwater environment (Hoar, 1988). Both spring and fall chinook 're-smoltify' in the autumn (Ewing *et al.*, 1979; Franklin, 1989). Seasonal changes in the environment, principally photoperiod and lunar cycles (*Oncorhynchus*), fine tune the endogenous rhythmicity (Ewing *et al.*, 1979; Wedemeyer *et al.*, 1980; Clarke *et al.*, 1981; Eriksson and Lundqvist, 1982; Grau, 1982; Grau *et al.*, 1982).

Various hormones are essential for parr-smolt transformation. Thyroid hormones (L-thyroxine, T_4 and 3,5,3'-triiodo-L-thyronine, T_3) co-ordinate, but do not trigger, the processes involved (Barron, 1986; Hoar, 1988). Prolactin, growth hormone and the anterior-pituitary corticotrophs, are elevated during transformation. The former is vital for sodium retention of the smolt in freshwater; the latter have important roles in hydromineral regulation at sea (Clarke and Nagahama, 1977; Nishioka *et al.*, 1982; Prunet and Boeuf, 1985). The corticosteroids, especially cortisol, and the hormones of the interrenal gland, are necessary for parr-smolt transformation, causing alterations of intermediary metabolism, and in some way elevating (synergistically with growth hormone) gill adenosine triphosphatase activity (Na^+-K^+-ATPase; Sheridan, 1986; Franklin, 1989; Madsen, 1989, 1990a,b,c).

Osmotic and ionic regulation of smolts differs from that of parr, with the former able to maintain electrolyte homeostasis in saline environments. The hypo-osmotic freshwater environment requires the excretion of large amounts of water, and acquisition of salts; the hyper-osmotic marine environment demands rigid conservation of fluid, drinking of seawater, and the excretion of salt. Many tissues are actively involved with these functions in the smolt, including gills, opercular epithelia, kidneys, urinary bladder, and intestinal epithelia. Gill Na^+-K^+-ATPase activity (Zaugg and McLain, 1970) increases during the parr-smolt transformation, reaching a maximum at the time of peak downstream migration and seawater entry (Zaugg, 1989; Zaugg *et al.*, 1985). Intestinal Na^+-K^+-ATPases also increase during

seawater adaptation (Oide, 1967; Jampol and Epstein, 1970; Pickford *et al.*, 1970; Epstein *et al.*, 1980).

Morphological changes are observed during parr-smolt transformation, especially in the 'trouts' (*Salmo spp*); smolt are silvery, and leaner than parr (Hoar, 1939; Vanstone and Markert, 1968; Fessler and Wagner, 1969). The length of the caudal peduncle, in proportion to fork length, changes in Atlantic (*Salmo salar*), coho (*Oncorhynchus kisutch*), and chinook (*O. tshawytscha*) smolts, compared to parr (Nikolskii *et al.*, 1947; Winans, 1984; Winans and Nishioka, 1984). Condition factor and proximal composition of Atlantic salmon parr and smolts differ, due to a decrease in total body lipid (Komourdjian *et al.*, 1976b). Sheridan and co-authors (1985b) have shown that this decrease is due to elevated lipolysis, and reduced lipogenesis. The fatty acid composition alters during parr-smolt transformation, with long-chain, polyunsaturated lipids predominating in the smolt (Lovern, 1934; Sheridan, 1985, 1986, 1989; Sheridan *et al.*, 1983, 1985a). The physiological, adaptive significance of this change is discussed by Hoar (1988, and others, cited therein).

The 'silvering' of smolts is due to deposition of hypoxanthine and guanine (purine) crystals within the skin (Vanstone and Markert, 1968; Johnston and Eales, 1967, 1968, 1970). This process is reversible if the smolts do not reach seawater, *i.e.*, 'post-smolts', if retained in freshwater, will recover the typical cryptic 'parr marks' by the resorption of the purines (these fish are said to have 'desmoltified', and are called 'parr-revertants': Mahnken, 1973; Folmar *et al.*, 1982; Mahnken *et al.*, 1982). Silvering is under endogenous control (Johnston and Eales, 1970), and is an important adaptation for a pelagic marine life. However, it may have evolved as a route of purine nitrogen metabolism (Hoar, 1988).

The behaviour of the smolt is different to that of the fry and parr (Schreck, 1982a). The smolt loses the rheotactic, stream-bed-oriented, and aggressive territory defending behaviour characteristic of parr, and assumes a more pelagic habit. Coupled with this tendency to remain in the water column proper, is the increased association with sibling fish within relatively large aggregates (schools). The timing of this behavioural change is often coincident with seasonal freshets which tend to 'flush out' or stimulate downstream displacement of the fish (Schreck, 1982a; Youngson *et al.*, 1983).

The evolution of the parr-smolt transformation has co-ordinated a series of unrelated physiological modifications that collectively pre-adapt young salmonids for the marine environment (Hoar, 1988). Though all the changes may be observed in wild fish, intensive hatchery rearing may alter this synchrony. This has meant that the production of fully transformed smolts is variable between hatcheries and between years, such that diagnostic tests of complete smoltification are necessary prior to large scale release of hatchery fish.

SEAWATER TRANSFER OF CHINOOK SALMON

Salmonids are commercially cultivated, and their wild runs enhanced in all areas of the world where they exist (Childerhose and Trim, 1980). Salmon farming, the propagation of fish to

market or adult size, is a relatively recent venture. There are essentially three methods by which salmon may be cultivated, and all involve a period of hatchery rearing.

Ocean ranching practices release smolts into rivers, and allow most somatic growth to occur in the ocean. The fish are then harvested as they return to the rivers of release on the spawning migration. Sea-cage rearing involves the production of smolts in freshwater, with subsequent on-growing achieved in net-pens. The position of such facilities are presently restricted to near-shore, coastal areas, that have a good tidal circulation. Salmon may also be farmed wholly within freshwater, although this practice is limited, in the main, to New Zealand.

Both ocean ranching and sea-cage rearing require the large scale production of smolts, and their successful transfer to seawater. Due to the fluctuation in seawater tolerance during the developmental stages of fry, parr, smolt and post-smolt (Weisbart, 1968), timing of release or transfer is of extreme importance. A simple diagnostic, or routine method for determining smolt status is required for the farming industry. Many assays exist that measure enzyme activities and other biological parameters of processes involved in osmoregulation, however these tend to be complicated laboratory based techniques that are not readily applied.

Despite the morphological changes associated with parr-smolt transformation, the presence of a silvery integument is not definitive of full smolt status, nor is a drop in condition factor, particularly with regard to hatchery reared fish (Folmar and Dickhoff, 1980; Gorbman *et al.*, 1982; Zaugg, 1982b,c; Langdon, 1985; Schreck *et al.*, 1985; Virtanen, 1987). Ewing and Birks (1982) examined indicators of parr-smolt transformation in spring chinook and found that only gill Na^+-K^+-ATPase activity was correlated with the timing of ocean entry. However, the same group of workers have also published work where gill Na^+-K^+-ATPase activity of chinook smolts did not predict maximum adult returns (Ewing, Fustish *et al.*, 1980; Ewing, Pribble *et al.*, 1980; Ewing and Birks, 1982; Ewing *et al.*, 1985).

Seawater challenge tests to determine the hypo-osmoregulatory ability of hatchery reared coho and chinook salmon were developed by Clarke and Blackburn (1977, 1978). The principal of such tests involved challenging the osmoregulatory capacity of 'smolts' over a 24 hour transfer to seawater. Smolts regulated plasma sodium within 24 hours of transfer, whereas parr did not (Clarke and Blackburn, 1977). Although common in the Northern Hemisphere, seawater challenge tests are not routinely used as part of the salmon rearing process in New Zealand. Langdon (1985) argues that although short term survival of hatchery smolts during a seawater challenge test may well prevent acute deaths, such a test does not predict long term marine survival of smolts released for ocean ranching (Ewing *et al.*, 1980; Brannon *et al.*, 1982; Clarke, 1982; Ewing and Birks, 1982; Mahnken *et al.*, 1982; Ewing *et al.*, 1985).

Franklin (1989) described physiological changes occurring in chinook following direct seawater transfer. Plasma chloride and osmolality remained elevated during seawater growth, compared to initial freshwater values. Cortisol concentration increased, returning to pre-transfer levels within 12 hours, during successful transfers, but remained elevated if the fish failed to adapt to seawater. This study expands and extends the previous work of Franklin (1989), and describes a routine osmoregulatory test for chinook (*see* CHAPTER 5, *48 hour seawater challenge*

test). The effect of salinity and laboratory rearing on growth and growth rate of underyearling chinook is dealt with in the sixth chapter (*see* CHAPTER 6, *salinity, improved trophic opportunity and growth*), with reference to optimal size at transfer, and the relative importance of size, age and timing of transfer.

Chapter 7 (*precocious parr*) describes the occurrence of two precociously mature male parr, that were found during the studies. The final chapter collectively discusses the results of this study, and draws some conclusions from the work (CHAPTER 8, *general discussion and conclusions*). As much of the methodology involved in this study was common to all the chapters, it was decided to collate a fully detailed account of all the methods and materials in a separate chapter (CHAPTER 2, *general methods*). Immediately following the last chapter are the acknowledgements I wish to make toward the many people that have made this thesis possible, and more importantly, exceedingly enjoyable. A comprehensive list of all the literature referred to in this study has been arranged into a single reference and bibliographical section, and is found at the back of the thesis.

EXERCISE TRAINING OF FISH

There have been many studies investigating the effects of exercise training on fish physiology. Davison (1989) reviewed the literature, and drew comparisons between changes known to occur in mammals, as a result of exercise training, with those found in teleost fish. Studies involving exercise training as an experimental tool, have been common in the recent past, due to parallels with sports medicine. In mammalian, and particularly human research, exercise studies have strict 'rules', regarding the type of exercise undertaken, the intensity and duration of each bout, and the parameters measured (Clausen, 1977; Borer, 1980; Blomqvist and Saltin, 1983). This rigorous regulation permits comparison between studies. Such regulation is not apparent in fish studies, and whilst fish may only be exercised by swimming, the means by which this is achieved are many. In addition, the physiological parameters measured vary considerably, which largely prevents direct comparison (Davison, 1989).

The effect of exercise training on growth has been variously studied on a number of teleost species. Salmonids have been used extensively in this research due to their economic importance. Davison and Goldspink (1977) were the first to demonstrate that exercise will promote growth in the salmonids; maximum growth was achieved with fish forced to swim continuously at 1-1.5 $bl.s^{-1}$ (body lengths per second). The fish used were yearling brown trout (Davison and Goldspink, 1977). There have been many other studies reporting similar results; fry and underyearling Arctic charr (Christiansen *et al.*, 1989; Christiansen and Jobling, 1990); underyearling rainbow trout (Nahhas *et al.*, 1982a; Davie *et al.*, 1986); yearling rainbow trout (Greer-Walker and Emerson, 1978); yearling brook trout (Leon, 1986); post-smolt and sub-adult Atlantic salmon (Kuipers, 1982; Totland *et al.*, 1987). The research has shown that exercise training may be a viable means to increase growth rate in the intensive farming of salmonids (Kuipers, 1982; Leon, 1986; Totland *et al.*, 1987). However, ration level is important to ensure

good growth when salmonids are exercised (White and Li, 1985). 'Sprint' training and its effect on growth has not been widely studied; however, in one study, it was found to be deleterious in terms of growth rate (Gamperl *et al.*, 1988; Gamperl and Stevens, 1991).

Prior to start of this study, there was no published work dealing exclusively with the effect of exercise training on growth rate of chinook salmon. It was decided therefore to investigate whether the growth rate of young-of-the-year chinook could be enhanced by training. White and Li (1985) had shown previously that it was important to maintain a high ration level to obtain good growth in chinook. Therefore, in this study, all fish were given an *ad libitum* feeding regime during growth and training experiments. From previous research on chinook, it is known that this species will 'smoltify' during the spring and autumn of the first year of life (Ewing *et al.*, 1979; Clarke and Blackburn, 1977, 1978). Size is an important parameter for seawater survival as it appears that a 'critical size for smolting' exists in this species (Clarke and Blackburn, 1978; Ewing *et al.*, 1979; Clarke, 1982; Franklin, 1989). Accordingly, a means to enhance the growth rate of chinook fry and fingerlings would be advantageous for the early seawater transfer and mariculture of chinook (*see* CHAPTER 3, *exercise and growth*).

MEASUREMENT OF SWIMMING PERFORMANCE

Swimming performance has been determined in a number of fish species using a variety of means, both in the wild and in the laboratory. One such technique is measurement of the critical swimming speed, U_{crit}, first described and employed by Brett (1964). U_{crit} values may be presented as either a real (centimetres/metres per second) or relative (body lengths per second) velocity. Real speed U_{crit} tends to increase, whereas relative speed U_{crit} decreases with (and as a function of) fish size (Webb, 1971a,b; Webb and Johnsrude, 1988; Webb and Weihs, 1983). Determination of U_{crit} gives a measurement of the maximum velocity fish can sustain over a prolonged time period. U_{crit} values are determined by interpolation for those fish that do not tire exactly at the beginning or end of a prescribed period. U_{crit} has been measured for a number of fish species (reviewed by Beamish, 1978; Davison, 1989). The methodology of measurement vary considerably due to the apparatus used and the objectives of each particular study (Beamish, 1978). In this study, Blažka type fish respirometers (Blažka *et al.*, 1960; Beamish, 1978) were used in the determination of U_{crit}.

Studies have revealed that salmon swimming ability (measured using U_{crit} procedures) varies during the few first months of life, with two periods of decreased performance during early riverine life. Swimming performance of alevins increases during development but shows a transient decrease upon complete yolk sac absorption (Thomas *et al.*, 1969). As this drop in performance coincides with the period of emergence, it has been proposed as a cause for the downstream migration of large numbers of fry (Thomas *et al.*, 1969). Swimming ability of young salmon improves during growth thereafter, until the period of parr-smolt transformation (Glova and McInerney, 1977; Thorpe and Morgan, 1978b; Flagg and Smith, 1982; Flagg *et al.*, 1983), when a decline has been observed. Furthermore, this reduction has been linked to the

passive, downstream, smolt migration. Smolts migrate downstream tail first (Thorpe *et al.*, 1981; Smith, 1982), actively swimming against the current, but at a slower speed than the water flow. In conjunction with the changes in behaviour at parr-smolt transformation, the loss of swimming ability would 'assist' in the overall displacement of fish downstream, and through the tidal environment of the estuary (McCleave, 1978). Chinook, unlike sockeye, have not been used in many U_{crit} studies. Part of this study examined the swimming ability of underyearling chinook and sockeye salmon. The effect of exercise training prior to the measurement of swimming performance was assessed. Swimming performance was measured on all stages of development from 'zip up' fry to post smolt fish (*see* CHAPTER 4, U_{crit} - *critical swimming speed*).

SUMMARY OF THESIS OBJECTIVES

Growth and development of underyearling chinook salmon, *Oncorhynchus tshawytscha*, with respect to exercise training and seawater ability will be investigated in this thesis.

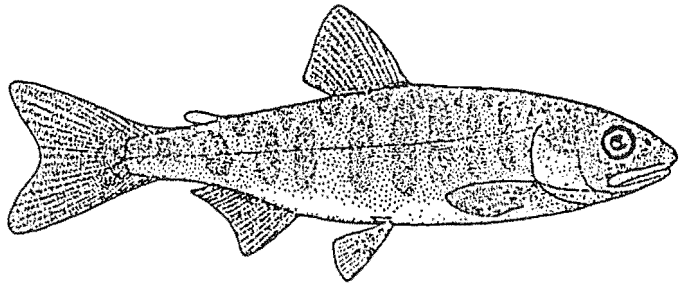
One major objective is to assess the period of parr-smolt transformation using seawater transfer experiments. From these investigations, it is hoped that a seawater challenge test, readily applicable to the New Zealand fish farming industry will be developed. Seawater adaptability will be assessed using percent survival, length and weight changes during the test, gill Na^+-K^+-ATPase enzyme activity, and subsequent seawater growth. Changes (morphological and physiological) associated with growth and development in freshwater and marine environments will be compared.

Another major objective of this thesis is to compare the growth and growth rate of exercise trained and untrained (both freshwater reared and seawater resident) chinook. Exercise training of the young salmon will take the form of low intensity (1-1.5 body lengths per second), enforced swimming in freshwater. Growth performance of these fish will be compared and contrasted to that of untrained (still water) fish held in freshwater and also seawater resident fish. Growth performance of 'accelerated' fish transferred to an 'improved trophic opportunity' environment will also be analysed and compared, relative to commercial hatchery-reared siblings.

Groups of exercise trained and untrained fish will be subjected to a critical swimming speed test (U_{crit}) to determine whether the training regime will confer a greater swimming ability on the young salmon. U_{crit} will also be measured for seawater resident fish and compared to the performance of freshwater siblings.

CHAPTER TWO

GENERAL METHODS



CHAPTER TWO

GENERAL METHODS

SALMON STOCKS

Chinook - hatchery fish

The majority of fish used in this study were underyearling (0+), hatchery reared chinook salmon (*Oncorhynchus tshawytscha*). All hatchery chinook originated from a Ministry of Agriculture and Fisheries' (MAF) experimental research hatchery, situated on the Glenariffe Stream, a tributary of the Rakaia River, New Zealand (All Government Departments, including MAF, have recently been rationalised, reorganised, restructured, and renamed. Currently, Freshwater Fisheries Research and Management is conducted within the new organisation called the National Institute of Water and Atmosphere, or NIWA). In all three seasons (season 1, 1989-1990; season 2, 1990-1991; season 3, 1991-1992) fish were derived from fertilized ova of sea-run adult chinook, returning to Glenariffe hatchery spawn in their third or fourth year (all two year old sea-run adults - mostly males - are not used for stock propagation).

Experiments for the first season were carried out at the Mahunga Salmon Farm, on the Kahutara River, Kaikoura, New Zealand. For seasons 2 and 3, chinook were obtained direct from Glenariffe and all experiments were performed at the Zoology Department, Canterbury University, Christchurch, New Zealand (hereafter the Department). On each sampling occasion, in each season, fish were obtained from the same raceway population to minimise variability. Salmon at Glenariffe are reared with a natural photoperiod, and fed to satiation daily under normal hatchery practices. The water temperature at Glenariffe hatchery varied over the year from a winter low of 6-7 °C, to a summer high of 11-13 °C.

Chinook - wild fish

During season 3, wild (*i.e.* naturally spawned) chinook were obtained from the Glenariffe Fry Trap. These fish were derived from wild sea-run parents, and prior to capture, had spent the early part of their life in the Glenariffe Stream. These fish were transported to the Department for experiments. The ages of the parents of these fish are unknown, as are their hatching dates.

Sockeye - hatchery fish

The sockeye salmon (*Oncorhynchus nerka*) used in this study were obtained from the Glenariffe hatchery. Originally taken from Larch Stream, a tributary of Lake Ohau, in the Waitaki District, sockeye have been held at Glenariffe since 1978. The sockeye were derived from fertilized ova of pond reared, landlocked adults, that were stripped in their third year.

SALMON COLLECTION AND TRANSPORTATION

Salmon were routinely transported in two-ply plastic refuse bags, placed inside 80 litre plastic

fish boxes. Two bags were used to minimise leakage of water into the box. The inner bag was filled with 20-30 litres of either bore (indoor, hatchery: alevin and fry stages) or Glenariffe Stream water (outdoor, raceway: fingerling, smolt and post-smolt stages). Salmon were quickly dip-netted from the troughs or raceways and placed into the bags, the time that the fish were in air being minimised. Oxygen was bubbled into the water and allowed to inflate the bag such that an 'oxygen rich reservoir' was present above the water. The bags were then sealed tightly with rubber bands to prevent the 'balloon' from deflating (in most cases, the bags were full on arrival at the Department). Fish were transported at a stocking density of 20-30 g per litre or less.

During the summer months, or on particularly warm 'nor-wester' days, ice and chilly-bin cooling pads were placed underneath and between the bags to keep the water cool. Fish were transported as early in the day as possible, and on most occasions arrived at the Department before midday. Survival of the fish was absolute, no fish died as a result of transport.

HUSBANDRY AND HANDLING

Freshwater system

On arrival at the Department, fish were dip-netted into aerated holding tanks, measured in groups of six, and placed into standard, 60 litre glass tanks, or the exercise flume (EXERCISE TRAINING, below). Each tank was supplied with artesian water, flowing at a rate of 1-6 litres per minute (increasing flow with fish size). Complete exchange of water occurred hour or less, depending on water depth. The freshwater supply was an 'open' system, such that water was not recirculated. Good aeration and circulation in each tank were achieved using air stones and compressed air. Plastic grilles were placed over each tank to prevent fish escape. Salmon were reared with a 12L:12D photoperiod. Freshwater temperature varied little (13-15 °C) during the experimental months.

Feed (commercial pellet diet - NRM Feeds, Nelson) was offered to the fish soon after placement in each tank. Usually, the fish fed within 24 hours of transport; the younger the fish, the quicker the resumption of feeding. Fish were fed, by hand, to satiation at least five times daily. Waste feed, bacterial build up, and faeces were removed as necessary by siphoning. In general, fish were only handled twice during each experiment, measurement at the beginning and end of each experiment. However some investigations (U_{crit} DETERMINATION and SEAWATER TRANSFER EXPERIMENTS, below) required repeated measurements.

Seawater system

The seawater set up at the Department is a closed, recirculating system. Seawater was set to flow into each tank at a rate of 4-6 litres per minute. Good aeration and circulation in each tank were achieved using air stones and compressed air. Plastic grilles were placed over each tank to prevent fish escape. Salmon were reared with a 12L:12D photoperiod. Seawater temperature varied slightly (13-16 °C) during the experimental months. Although the salinity of the seawater

varied from 28-38‰, during the course of the experiments, it was more stable on a day to day basis. In season 2, fish were transferred to 80 litre opaque (blue) plastic tanks. In season 3, transfers were to 80 litre glass tanks. All fish (except those undergoing 48 hour seawater challenge tests, below) were fed to satiation at least five times daily. Fish used in the challenge tests were not fed for 48 hours prior to transfer, nor during the first 48 hours in seawater. The seawater transferred fish were then fed to satiation as above.

ANAESTHESIA

Two fish anaesthetics were used during this study, and each was made to two strengths. Dilute anaesthesia was required for routine measuring, and a more concentrated solution was required for killing fish at the end of experiments. Phenoxyethanol was used during season 1 and initially during season 2 (4-6 ml, and 30 ml, 2-phenoxyethanol per litre for the dilute and concentrated solutions respectively). Benzocaine was used thereafter, as concentrated phenoxyethanol caused blood to clot in the gills of seawater adapted chinook (25-50 mg, and 300-500 mg, ethyl *p*-amino benzoate per litre for the dilute and concentrated solutions respectively). Benzocaine was first dissolved in approximately 10 ml, *n*-ethanol to aid its miscibility in water. The anaesthetics were prepared with both fresh and sea water. Fresh anaesthetics were made up on each sampling date.

Time to unconsciousness in dilute anaesthetic varied with fish size, but generally fish lost equilibrium within 30 seconds and were able to be handled within another minute. Concentrated anaesthetic killed fish within ten seconds. Deeply anaesthetised fish in the dilute anaesthetic were resuscitated by flicking the cardiac area of the thorax. Fish were always measured after they had been sedated in dilute anaesthesia.

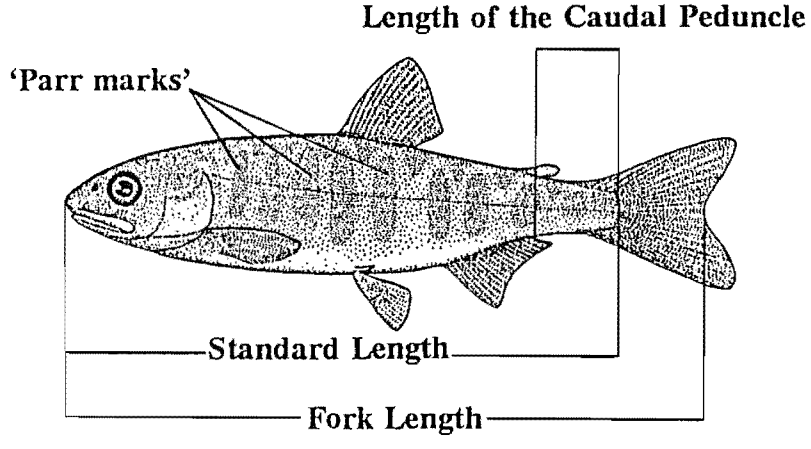


Figure 2.1 Schematic representation of a chinook fingerling (after McDowall, 1990b) with characteristic 'parr marks', showing measurements of fork length, standard length, and length of the caudal peduncle.

INITIAL MEASUREMENTS AND FISH MARKING

All fish were measured at the beginning of any experiment. Fish were lightly anaesthetised and then dip-netted onto a cold glass plate and measured for fork length (length to caudal fork) using Vernier callipers (accurate to 0.01 mm, Figure 2.1). The fish were then blotted dry on absorbent tissue before being weighed in a container of water (tared to zero on a Mettler PE360 balance, accurate to 0.01 g). In season 3, standard length and the length of the caudal peduncle were also measured (Figure 2.1).

In seasons 2 and 3, fish were marked individually. Colour dyes were used in season 2, with Alcian Blue proving the most successful when sparingly injected subcutaneously with a hypodermic syringe (20 Gauge × 1" needle, Herbinger *et al.*, 1990). Fish were marked ventro-laterally on the right and left side at the pectoral, pelvic and anal fins (Figure 2.2A). The dye remained in the skin and immediate musculature for at least a fortnight.

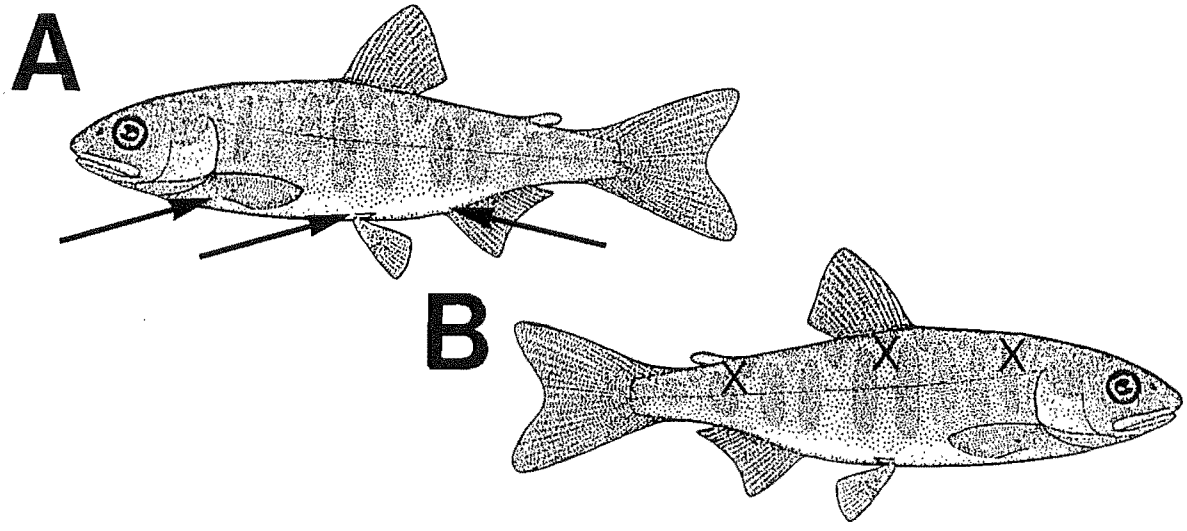


Figure 2.2 Schematic representations of chinook fingerling (after McDowall, 1990b) indicating where Alcian Blue dye was injected under the skin (Arrows, Figure 2.2A), and positions where the cold brands were applied (Crosses, Figure 2.2B). Both Alcian Blue dye, and the cold brands were applied to either side of the salmon. 'Dot' brands were applied to all fish less than 60 mm fork length; letter and symbol brands (C, I, T, V, X, and 45-180° branding iron rotations) were applied to bigger fish.

Cold branding, a quicker technique (Everest and Edmundson, 1967; Mighell, 1969; Raleigh *et al.*, 1973; Smith, 1973; Refstie and Aulstad, 1975), was used to mark fish in season 3. Five brands with wooden dowelling handles were made from one millimetre diameter brass welding rod, bent into the letters C, I, T, V, and X. These letters were chosen as it was possible, with 45°, 90°, and 180° rotations, to increase the total number of different markings and symbols. Brands were cooled in liquid nitrogen and applied dorso-laterally to the fish on the right and left sides, at three positions (Figure 2.2B). The brands were held against the skin

for one or two seconds. When fork length was less than 60 mm, 'dots' were applied using the tip of the I brand.

Fish were branded after they were blotted dry with tissue paper, prior to being weighed. Fish were branded once, and in one position only (although double and triple brands were occasionally used). The symbols were just visible soon after branding, and then disappeared until about 48 hours post branding, when they returned as dark, vivid marks. Brands were clearly visible for at least four weeks. Long term growth studies required re-branding of the fish at every re-measurement (fortnightly-monthly intervals).

EXPERIMENTAL DESIGN

The experimental design differed for each season as a result of practical experience. The design of the whole study was to monitor changes in the various parameters measured, throughout the first year of life. Fish were measured into groups of six (or more) for each control or experimental treatment. During season 1, a long term exercise training regime, with three different water velocity treatments, was devised to monitor growth rate of underyearling chinook at each training level. The logistical problems associated with the data collection of season 1, and the fact that it was not possible to undertake seawater transfer experiments (distance from seawater laboratory facilities) or critical swimming speed (U_{crit}) tests during season 1 (lack of mains power at the farm) necessitated the switch to the Department thereafter. Furthermore, as the results obtained from season 1 did not reveal a *positive* training effect, it was decided that during season 2 short term (fortnightly), continuous swimming, training regimes would be imposed on consecutive groups of sibling fish over the first growing season in order to determine whether training would have a beneficial effect at certain periods of development. During season 3, the training regime was further reduced to eight hours of swimming per day during the fortnightly training period. This step was taken as the continuous swimming regime similarly reduced the growth rate of trained fish.

Growth and growth rate

Growth and growth rate of the salmon was measured in each season. Growth rate of underyearling hatchery chinook was compared between Glenariffe hatchery fish (7-11 °C) and Departmentally reared fish (13-15 °C) in seasons 2 and 3. Similar comparisons of growth were made between sockeye salmon reared at the Department and those that remained at Glenariffe. Changes in body (standard length and caudal peduncle length) and tissue (heart and viscera weight) morphometry were followed. Growth rate was measured in terms of both the change in length and weight. The growth of fry, fingerling and smolt were measured over consecutive 13 day periods during the first eight months of life.

Linear growth rate (LGR, increase in millimetres per day) was calculated with the following equation, where L_f and L_i are the final and initial fork lengths of the fish respectively, and *time* is the number of days between measurements.

$$LGR = \frac{L_f - L_i}{time}$$

The weight specific growth rate (SGR, percentage increase in body weight (gram) per day) was calculated with the following equation

$$SGR = \left[\frac{ln(W_f) - ln(W_i)}{time} \right] \times 100$$

where $ln(W_f)$ and $ln(W_i)$ are the natural logarithms of the final and initial weights of the fish respectively, and *time* is the number of days between measurements.

Condition factor was calculated for each fish. Weight at any length, and *vice versa*, is highly variable between fish in any given population. Condition factor reduces this variability and allows for meaningful comparisons between groups especially over short time periods. There are several ways of computing fish condition factors (Le Cren, 1951; Bolger and Connolly, 1989; Cone, 1989). The equation used was the 'salmonid' condition factor (Frost and Brown, 1967):

$$k = \frac{W \times 100}{L^3}$$

where W is the weight (in grams), and L is the fork length (in centimetres) of the fish. This equation is often also called 'Fulton's condition factor' (Fulton, 1904; Frost and Brown, 1967).

Exercise training

The effect of exercise training on growth and swimming performance was measured in each season. Three different exercise training regimes were imposed on the young salmon. The design of the experiment run during the first season was to determine what effect long term, continuous duration exercise had on the growth rate of underyearling chinook salmon. The experimental design of seasons 2 and 3 monitored the effect of shorter term exercise training on growth. In season 2 fish were swum continuously, whereas in season 3, the swimming period was reduced to 8 hours a day. Additionally, swimming ability (CRITICAL SWIMMING SPEED - U_{crit} below) was compared between exercise trained and control fish.

Season 1: - long term (30-85 day), continuous swimming

In season 1, the training regime involved long term (30-85 day), continuous duration swimming. A four lane fish race was constructed (marine grade plywood, Figure 2.3) and positioned within a salmon raceway at the Mahunga Salmon Farm, Kaikoura (situated on the Kahutara River, 42°,30'S, 172°,30'E). Each lane was identical (15 × 15 × 200 cm), with retaining screens (plastic netting) set 150 cm apart, forming the swimming compartments. Inflow to each lane was regulated by adjusting the size of the inflow aperture from the header tank. Outflow was

regulated by using perspex boards at the end of each lane. These boards had varying numbers of 1-1.5 cm diameter holes drilled through them, the holes increased the flow through the lane. The water depth was held constant in each lane at 10-12 cm. Flow rate was maintained by a pressure head in the header tank. A large wooden board afforded the salmon cover, and plastic netting, secured over the entire construction, prevented fish escape.

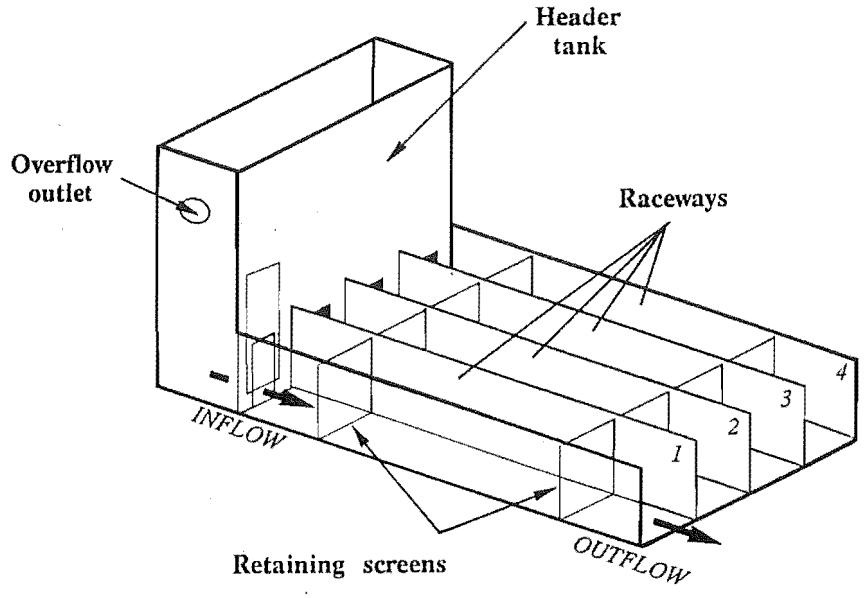


Figure 2.3 Representation of the four lane raceway used during season 1. The overflow outlet in the header tank ensured that a constant pressure head was maintained, and therefore that the flow rates through each lane were constant. The three exercise training lanes had flow rates of 5.2. 8.9, and 12.9 $cm.s^{-1}$ throughout the study. The 'control' (untrained) lane also had a constant flow rate of 1.3 $cm.s^{-1}$ throughout.

The design of the race was similar to that used by Nahhas and co-workers (1982a), and permitted the setting of different flow rates in each lane. The flow rates were set to represent initial velocities of 0.25, 1.0, 1.7 and 2.5 body lengths per second ($bl.s^{-1}$), corresponding to 1.3, 5.2, 8.9 and 12.9 $cm.s^{-1}$. The fish in the slowest lane (1.3 $cm.s^{-1}$) were used as 'still water', untrained controls. Water speed was determined for each lane by the following method. The time taken for each lane outflow to fill a 22 litre bucket was measured. Water velocity ($cm.s^{-1}$) was then calculated by dividing the volume flow ($cm^3.s^{-1}$) by the surface area (cm^2) of water travelling down the race.

Forty underyearling chinook were measured for fork length and weight, and were placed into each lane at the start of the experiment (6 October 1989). Flow rates were reduced following placement, and were increased when the fish were observed to be taking feed (normally within 60 minutes). The fish were forced to swim continuously for up to 85 days, and were fed every 30 minutes during the hours of daylight by automated feeders. The fish were reared under the natural photoperiod for the latitude. Fish were periodically remeasured for fork length and weight (25 October, 10 and 29 December 1989). During each such sampling, ten fish were killed for analysis (SAMPLING, below). Surviving fish were returned

to the same lane and allowed to recover from the stress of handling in reduced water flow, as before. The initial flow rates were kept throughout the experimental period, and therefore swimming speed, in terms of body lengths per second, decreased with fish growth.

Season 2: - short term (ten day), continuous swimming

In season 2, the training regime involved short term (ten day), continuous duration swimming. A 175 litre oval, racetrack flume (constructed in marine grade plywood, Figure 2.4) was utilised. The width of the single racetrack lane was 14 cm, the bends followed an outer diameter of 28 cm (inner, 14 cm), and were connected by two stretches, 140 cm long (the height of the lane was 30 cm). The 'swimming compartment' was the length of one of the long stretches. Stainless steel screens were positioned at either end of the compartment; the downstream one being electrified (0-50 volt, DC). Plastic netting was secured over the compartment. Fish could be viewed from above, or side on, through perspex windows.

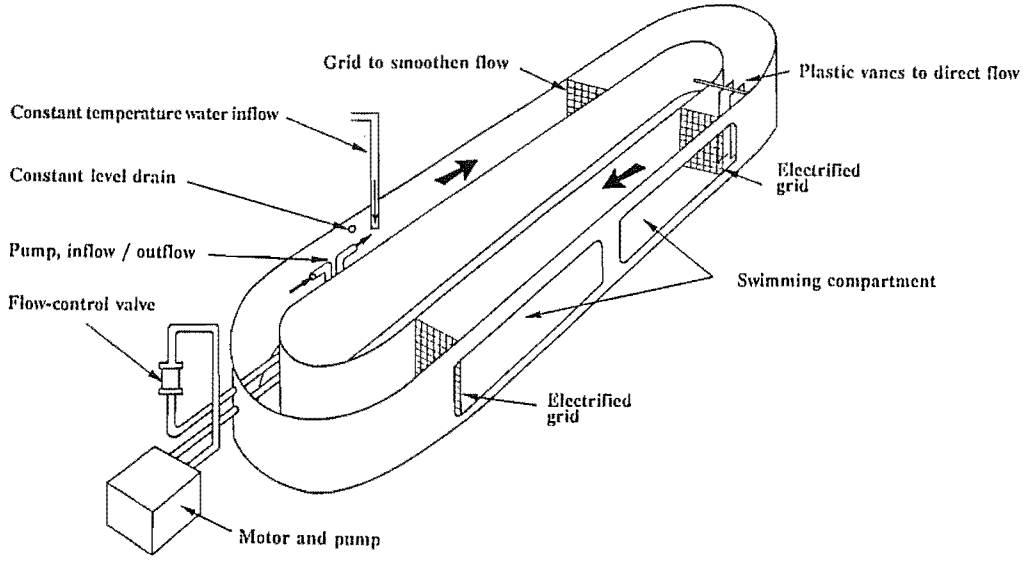


Figure 2.4 Representation of the oval, racetrack flume used for exercise training experiments of season 2 and 3. The 'swimming compartment' is indicated, as are the water positions of the perspex vanes that directed and effected rectilinear flow. Water flow was produced by a 'Davies SP' motor pump, the water inlet to and outlet from the pump are also shown (This diagram is modified from that drafted by Mr GT Robinson, a Zoology Department technician).

Artesian water was supplied at the rate of 5-10 litres per minute, ensuring complete water exchange every 15-30 minutes depending on water depth (20-23 cm, corresponding to 115-132 litres of water). Good aeration was ensured by gently bubbling compressed air through air stones. Water flow was produced by a 'Davies SP' type motor driven pump. The water velocity could be altered with a tap valve that restricted water flow into the pump. Actual water velocity was altered at the beginning of each trial to ensure that in all the trials, the fish were required to swim at a relative speed of 1-1.5 $bl.s^{-1}$ (water velocity was determined using a Kent

mini-flow probe). Rectilinear flow was achieved using various plastic vanes and grids at the pump outflow, and at the head of the bend leading into the swimming compartment. Heat generated by the pump was counteracted by cooling the artesian water inflow. The temperature of the water was regulated by a cooling unit and was set to match that flowing into the glass tanks (*i.e.* those used to rear the untrained fish).

During the season (2 November 1990 to 15 March 1991), groups of six fish were measured for fork length and weight, and transferred to the flume (exercised trained group) or to a glass tank (untrained group). Trained fish were allowed at least 48 hours to recover from handling and transportation, and were then forced to swim continuously against a current of 1-1.5 $bl.s^{-1}$ for ten days. All fish were fed to satiation at least five times daily. Water flow was not stopped during feeding. Seven trials of this nature were repeated on different groups of fish over the course of season 2.

Season 3: - short term (ten day), periodic swimming (eight hours per day)

In season 3, the training regime involved short term (ten day), periodic (eight hours per day) swimming in the flume. The fish swam during the hours of daylight (9 a.m. to 5 p.m.). As with season 2, sibling groups of six chinook were forced to swim against a current of 1-1.5 $bl.s^{-1}$. Consecutive groups of hatchery chinook were trained in this way every fortnight from 28 June to 24 October 1992. Fish were fed at least five times daily; before, during and after the period of training. One group of six wild 0+ chinook smolts was exercise trained in the flume. A group of six underyearling sockeye was also subjected to training in this way. Growth in both of these groups was compared to that attained by untrained fish reared in 'still water' (*i.e.* within 60 litre glass tanks, as in season 2).

Swimming performance - determination of the critical swimming speed (U_{crit})

Two Blažka-type fish respirometers were used for the measurement of U_{crit} (Blažka *et al.*, 1960). The use of each depended on fish size. Fish heavier than 30 g were tested in the bigger device. The construction and use of both was essentially the same and therefore only the workings of one will be described below. The Blažka respirometer design basically employs a motor driven impeller to pull water through a cylindrical swimming chamber or 'tunnel' (Figure 2.5). The material used in the construction of the respirometer was clear perspex unless stated otherwise. The respirometer was a rectangular box (45 × 14 × 18 cm) with concave baffles at each end and a tight fitting lid. The impeller (8.5 cm, external diameter; fibreglass) was mounted through an end wall into a cylindrical casing (10 cm, internal diameter).

The swimming tunnel (9 cm internal diameter, 10 cm external diameter, 31 cm long) slotted tightly into the casing and was secured at the other end by a foot piece. Vanes (3.5 cm long) and a stainless steel grid, at the 'upstream' end of the tunnel reduced turbulence and effected rectilinear flow. An electric screen (stainless steel wire) was built into the impeller casing and connected to a microscope rheostat (0-14 volt, DC). The steel grid and the electrified screen effectively formed barriers to the salmon. The length of the tunnel available to the fish

for swimming was 28 cm. The impeller was belt driven by a series of four pulleys (gears) and a variable speed control. A Kent mini-flow probe was used to calibrate the water speed at each setting on the variable control, and for each gear. Water flow speeds of 5 to 105 cm.s^{-1} were possible with both machines.

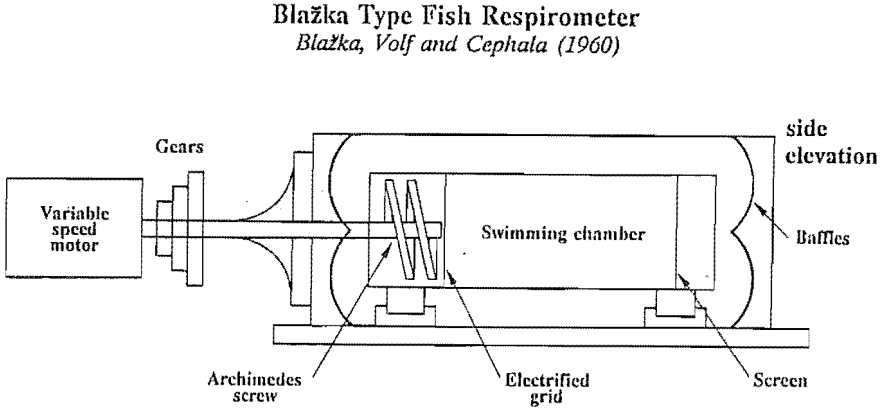


Figure 2.5 Representation of a Blažka-type respirometer used to measure the critical swimming speed (U_{crit}) of young salmon. The respirometer box was constructed of 10 mm clear perspex, the 'swim tunnel', impeller casing, and baffles of 5 mm clear perspex. A stainless steel screen at the upstream end of the tunnel, and an electric grid (0-14 volt, DC), wired into the upstream end of the impeller casing were complete barriers to fish escape. The concave baffles and the vanes effected rectilinear flow through the tunnel. Water flow was produced by the revolutions of the impeller, attached to a one horsepower motor. All water speeds from 5-105 cm.s^{-1} were possible with a gearing system and a variable speed control.

Routine measurement of U_{crit}

All the U_{crit} tests were performed with only a single fish in the swim-tunnel at a time. Tests were performed in both freshwater and seawater. Seawater tests were performed on fish that had been resident in seawater for at least a week. Fish were dip netted from either the glass tank or the flume, lightly anaesthetised and measured as before. During recovery, they were placed into the 'tunnel', and this was secured to the impeller casing. The respirometer was filled with water and the lid tightly sealed. A 'sampling port' in the lid was left open and therefore the water was fully oxygenated during the U_{crit} tests. A mirror was positioned at a 45° angle on the top of the lid, and facilitated fish observation. The fish was allowed to recover for at least 10 minutes prior to the start of the test. During this time, the fish regained balance, and explored the confines of the tunnel. Water velocity increments in terms of body lengths per second (bl.s^{-1}) were calculated from the fork length, and converted to the appropriate gearing and variable speed control settings.

To start the test, a water velocity equivalent to one bl.s^{-1} was set, and the fish were required to swim against this current for 10 minutes. A low voltage (less than two volts, DC) was set across the electric grid during the test to encourage the fish to swim (this was not possible during seawater U_{crit} tests due to electrolysis and salt water conductivity). Step wise

increments of one $bl.s^{-1}$ were imposed on the fish every five minutes thereafter, until the fish fatigued. This methodology differs somewhat from the 10 cm increments and one hour duration time periods suggested by Brett (1967) and Beamish (1978), but was chosen due to the size range of the fish to be studied and time constraints if all the fish in each trial were to be tested. Fish were observed throughout each trial, to ensure continuous swimming, and whether swimming was effected by steady or unsteady movements. Ventilatory rate (gill opercula movements) was determined before and immediately after the test, and was counted in two separate, 10 second periods during the last 30 seconds of each velocity increment.

Exhaustion was observed when the fish failed to maintain its position in the current, being forced backwards on to the screen. If increased electrical stimulation (14 volt DC, maximum) failed to force the fish to continue swimming, the test and the water flow was stopped. Exhausted fish would lie on the screen, or on the 'floor' of the tunnel for up to 30 seconds, ventilating deeply. After this time, they would regain their balance, and rest on the tunnel floor. In some instances, fish 'faked' fatigue, and showed an unwillingness to swim early in the U_{crit} test. Such fish did not behave in the same way as truly exhausted fish. Although repeated electrical stimulation failed to remove them from the grid, as soon as the water current was stopped, the 'fraudster fish' would immediately regain a stationary position on the tunnel floor, or gently swim around in the tunnel. Upon noticing such behaviour, the flow was immediately restarted. In some cases, the fish would return to the downstream grid within 5-10 seconds, and if this happened the trial was terminated. If, however, the fish took up steady swimming again, the trial was continued. At each subsequent failure, these fish were similarly checked, until exhaustion was evident. The test methodology was similar to that of Smit and co-workers (1971) in this regard. At the conclusion of each test, fish were quickly removed from the respirometer, killed by overanaesthesia, remeasured and dissected (sampling, below). The respirometer was fully drained and flushed with fresh water for several minutes. It was then three-quarters refilled and the water gently aerated with compressed air bubbling through air stones.

Most fish did not tire exactly at the beginning or end of a prescribed swimming period. For those fish that fatigued during the five minute intervals, the critical swimming speed was calculated by interpolation, with the following formula (Brett, 1964)

$$U_{crit}\ (\textit{critical swimming speed}) = v_p + (t_e/t_i \times v_i)$$

where v_p is the penultimate velocity, *i.e.* the fastest velocity that was maintained for the whole of the prescribed swimming period; t_e is the time elapsed prior to fatigue at the ultimate velocity; t_i is the duration of the prescribed swimming period; and v_i is the velocity increment. With this equation, the values for v_p and v_i are interchangeable with regard to the real swimming speed ($cm.s^{-1}$) and the relative swimming speed ($bl.s^{-1}$) of the fish.

Four fish were subjected to 'multiple' U_{crit} tests to determine whether there was a 'conditioning' effect on critical swimming speed. Each fish was tested individually and on three

occasions. At the end of a U_{crit} test, the fish was netted from the respirometer and placed into a recovery tank for a period of four hours - previously shown to be a suitable length of time to permit full recovery to a 'resting' state (Professor AP Farrell, personal communication, 1991). After all four fish had been tested once, the first of the group was returned to the respirometer box and subjected to the second U_{crit} test. The fish were treated in this way until three separate measurements of U_{crit} had been determined for each fish. Physiological analyses were not performed on these fish.

SEAWATER TRANSFER

All fish that were used for seawater transfer experiments were allowed to recover from the stress of transport, in freshwater holding tanks for at least 48 hours after arrival at the Department. During this time they were not fed, to allow the gut to empty. On arrival they were lightly anaesthetised and individually cold branded (season 3 only).

Forty-eight hour seawater challenge test

After the recovery period, the fish were individually measured for fork length and wet weight. Once measured, the fish were allowed to recover in aerated freshwater for at least 30 minutes prior to seawater transfer. All transfers were performed between 10 a.m. and 12 noon. The fish were transferred directly into full strength seawater ($\approx$30‰, 80 litre tanks) by dip netting.

The seawater system in the Department is a closed, recirculated system. Seawater was set to flow into each tank at a rate of 4-6 litres per minute. Whilst in the Department, the salmon were reared under a 12L:12D photoperiod. The seawater temperature varied slightly (13-16 °C, in line with the seasonal changes of freshwater temperature, 13-15 °C), during the experimental months, as did the salinity (28-38‰). Salinity was far more stable on a day to day basis. In season 2, fish were transferred to 80 litre opaque (blue) plastic tanks. In season 3, transfers were to 80 litre glass tanks.

Fish were observed immediately after transfer, and thereafter every 30-60 minutes over the next 48 hours. During the twelve hours of 'night', dim light from the corridor running along side the Aquarium Room permitted the fish to be viewed without startling them. General fish behaviour was noted at each observation. Dead fish, fish that had lost their balance equilibrium, and fish that were lying on the bottom of the tank and were unable to swim were removed (considered as good as dead), and the time of death noted. These fish were killed (if necessary) using a concentrated anaesthetic made up in seawater and were dissected. All the fish that survived the 48 hour seawater challenge test were remeasured at 48 hours post transfer. After remeasurement the fish were allowed to recover in seawater, and were then returned to the seawater tank, and fed thereafter at least five times daily to assess subsequent growth rate.

During season 3, six fish from the total pool of fish to be transferred to seawater were killed on the morning of a seawater challenge test to record initial, freshwater, resting levels of various physiological parameters (termed the 'initial group'). Similarly, at the 48 hour

remeasurement, a second group of six fish were killed for physiological analysis (termed the '48 hour group'), and provided an indication of the physiological status of the fish after 48 hours in seawater. Fish that died as a result of seawater transfer were collectively termed the 'dead' group. Actual and percentage loss of length and weight were calculated for individual fish at death, and/or after 48 hours survival in seawater. Weight loss over 48 hours in groups of 'freshwater control' fish held (and not fed) in freshwater was determined on a number of occasions during season 2 and 3.

Seawater adapted fish

Survival of seawater transferred fish was monitored after the 48 hour challenge test, and any dead fish were dissected (SAMPLING, below). The remaining fish were fed to satiation at least five times daily at the same time as the freshwater fish. Growth and growth rate of seawater adapted fish was followed thereafter. Seawater adapted fish were subjected to U_{crit} tests. It was unfortunately not possible to subject seawater adapted fish to exercise training, due to corrosion in the pump mechanism of the flume.

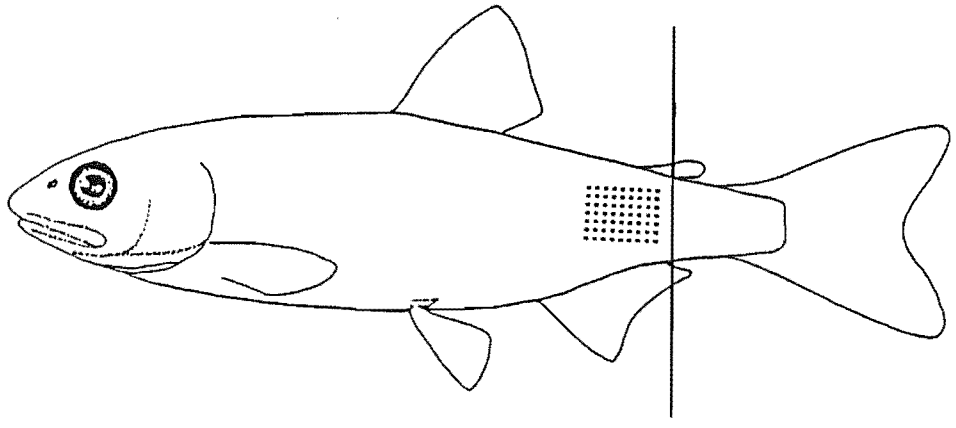


Figure 2.6 Line drawing of a chinook fingerling (after McDowall, 1990b) showing where the tail was severed between the adipose and anal fins. Blood was collected from the caudal vasculature at the tail stump. The sample of white muscle taken for percentage water content was taken immediately anterior to the cut, as shown by the dotted area.

SAMPLING

Fish were killed by overanaesthesia. They were measured (weight was determined by placing the fish on a moist paper towel - tared to zero on the balance - and not in water). The caudal peduncle was wiped free of mucus and was then severed with a scalpel immediately posterior to the adipose and anal fins (Figure 2.6). Blood was collected from the caudal vasculature into ammonium heparinised micro capillaries (1-100 μl). These were plugged with plasticine or '*Sigilium*' (Tradename) and centrifuged for six minutes at 5000 *g*. Haematocrit was determined

for each micro capillary, and averaged. The tubes were then cut and the plasma transferred to marked polyethylene vials (1.5 ml Eppendorf centrifuge tubes). The plasma was then frozen in liquid nitrogen. Individual fish were bled in this way within 3-5 minutes of capture.

A sample of white muscle was taken from the area immediately anterior to the original cut (dotted area of the tail stump in Figure 2.6). A scalpel blade was used to free the muscle tissue of skin, blood, and red muscle. The muscle sample was then wrapped in a piece of pre-weighed aluminium foil. The carcass was wiped clean of coagulated blood, and a mid ventral cut was made from the vent to the tip of the lower jaw. The heart (sinus venosus, atrium, ventricle, and truncus arteriosus) was located, removed, blotted dry of blood on absorbent tissue, and then wrapped in another piece of pre-weighed foil. The viscera (alimentary tract, swim bladder, liver, and gall bladder) were then removed and placed in a 'cup' of pre-weighed foil. These samples were immediately weighed, and wet heart and viscera weight calculated. These samples were taken for determination of tissue water content (see below).

The gill arches were removed, dissected free of connective tissue, rinsed in homogenising buffer (see below), placed into marked vials, and frozen in liquid nitrogen. Gills were stored at −80 °C before analyses of Na^+-K^+-ATPase activity were performed. A sample of the myotome was taken, or the whole fish, and frozen in liquid nitrogen. The plasma, gills, and the myotome sample were stored at −80 °C prior to analysis.

TISSUE ANALYSIS

Tissue water content

The foils containing the muscle, heart, and viscera were weighed on a Mettler AJ-100 balance, accurate to four decimal places (a Cahn 21, CAHN/Ventura Balance, accurate to 100 μg, was used when weighing hearts that were less than 10 mg wet weight). These tissues were then placed in an oven and dried to constant weight at 70 °C. Wet and dry tissue weights and percentage water content of each tissue were determined for each sample.

Plasma electrolytes and osmolality

Sodium concentration ($mmol.l^{-1}$) was analysed by atomic absorption spectrophotometry using a Varian Techtron 1200 Absorption Spectrophotometer. Duplicate 5 μl samples of plasma were diluted to a final volume of 5 ml with a 2 $g.l^{-1}$ KCl solution, and were immediately aspirated. The concentration of each sample was estimated from a calibration curve set up using NaCl standards, that were read before and after the samples (blank and standard solutions were made up using the 2 $g.l^{-1}$ KCl solution). Swamping the standards and samples with potassium, depresses sodium ionisation, and thereby prevents loss of accuracy. The flame was set transversely across the lamp beam to allow for the measurement of the relatively concentrated samples.

Chloride concentration ($mmol.l^{-1}$) was determined using a Radiometer CMT 10 Chloride

titrator. Duplicate 10 μl samples of plasma were titrated. Osmolality ($mOsm.kg^{-1}$) was determined using a Wescor Incorporated 5100C vapour pressure osmometer. Duplicate 8 μl samples of plasma were processed. Although plasma was collected from individual fish, it was pooled, as necessary, before analysis, in order that all three determinations could be carried out on each experimental group.

Determination of gill sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase) activity

A number of methods for determining gill Na^+-K^+-ATPase activity have been described in the literature (Zaugg and McLain, 1970; Jonhson *et al.*, 1977; Zaugg, 1982a; Langdon *et al.*, 1984). Regardless of technique, the fundamental principle of determining the specific activity of gill Na^+-K^+-ATPase is the same.

Determination of the entire gill ATPase activity (Na^+, K^+, and Mg^{2+}, -ATPase activities) is quantified by measuring the total amount of inorganic phosphate (P_i) released upon adenosine triphosphate (ATP) hydrolysis. Na^+-K^+-ATPase activity is calculated as the difference between total ATPase activity and that attributable to Mg^{2+}-ATPase alone (Na^+-K^+-ATPase enzyme activity is inhibited in the presence of ouabain). The specific activity of gill Na^+-K^+-ATPase is calculated as the difference in the rate of inorganic phosphate liberated in the presence and absence of ouabain, per milligram of gill protein, per hour.

The methods described in the literature differ primarily in the extent of enzyme purification. Many of the procedures require differential centrifugation and prolonged ultracentrifugation to separate interfering substances and to increase the purity of the enzyme fraction (Epstein *et al.*, 1967; Thompson and Sargent, 1977). In a review by Zaugg (1982a), the use of the purification methods was seen as being impractical when measuring the activity of a large number of samples. Without purification, crude homogenates of gill tissue cause a measurable reduction in Na^+-K^+-ATPase specific activity. However this drawback is balanced by the increased number of samples that can be processed. Additionally, the reduction in activity is constant for all samples. Therefore, in this study, crude gill homogenates were used in gill Na^+-K^+-ATPase activity determination. The assay methodology was based on that described previously (Johnson *et al.*, 1977; Langdon *et al.*, 1984; Franklin, 1989).

Frozen gills were thawed on a cold glass plate, and the gill epithelium scraped from the gill arches using a scalpel blade (with exceedingly small gills, the arches were separated and crudely 'chopped'). The gill filaments/epithelia were weighed and transferred to a glass homogenising vial, and suspended in approximately 40 volumes of cold homogenising buffer (100 mM imidazole, 10 mM Na_2EDTA, 10 mM 2-mercaptoethanol, 300 mM sucrose, pH 7.2; sucrose was added on the day of the assay). The filaments and epithelia were homogenised with the vial held in crushed ice, using a motorised teflon pestle set to 600 rpm for 2-4 seconds. The crude homogenate was allowed to settle for 30 seconds. Duplicate (A and B) 100 μl samples were taken from the upper half of the homogenate and transferred to 4.5 ml, perspex '3DT' tubes, sitting in a crushed ice water-bath. Care was taken at this stage to ensure a homogeneous sample was taken. Sizable, partially homogenised gill tissue fragments that tended to settle at

the bottom of the vial, were avoided. The 100 μl samples contained 60-200 μg protein. Each sample was then made up to 300 μl, with the addition of 200 μl of homogenising buffer. Duplicate ATP hydrolysis (for both A and B), homogenate blanks (300 μl homogenising buffer) were included in each assay.

Duplicate samples were taken to permit the simultaneous measurement of total ATPase activity in one tube (1.2 ml incubation medium A; 150 mM NaCl, 75 mM KCl, 20 mM $MgCl_2$, 100 mM imidazole, 10 mM Na_2ATP, pH 7.2), and Mg^{2+}-ATPase activity in the other (1.2 ml incubation medium B; as incubation medium A, but with the addition of 0.58 mM ouabain. Ouabain, and Na_2ATP were added on the day of the assay). After the incubation medium (A or B) was added, each tube was thoroughly vortex mixed. Samples were incubated for 30 minutes at 37 °C in a water bath, and then the reaction was quickly terminated by adding 300 μl of ice cold 10% trichloroacetic acid. Each tube was vortex mixed and placed in a −20 °C freezer for five minutes. Samples were then centrifuged at 3400 rpm for 30 minutes to pellet the protein.

The concentration of inorganic phosphate produced from the enzymatic hydrolysis of ATP was measured in 500 μl of the supernatant using the technique of Peterson (1978). In order to ensure rapid colour development of phosphate, the stock solution of ANSA (1-amino-2-naphthol-4-sulphonic acid) was made up fresh on the day of each assay, and was not diluted. The amount of protein in each pellet was determined using the method of Lowry and co-authors (1951) with the modifications of Hartree (1972) to increase linearity of the standard curve. Bovine serum albumin was used as the protein standard. Specific activity of gill Na^+-K^+-ATPase was expressed as micromoles inorganic phosphate released, per milligram protein, per hour (μmol P_i.mg protein^{-1}.h^{-1}).

DATA HANDLING

The spreadsheet, PLANPERFECT 5.1 (Wordperfect Corporation) package was used to calculate the various parameters measured above. Figures were created with FIG P. 5.1 (Fig P. Software Corporation) or drawn free hand. Statistical analyses were carried out using STATISTIX 3.1 (NH Analytical Software). The document was written and formatted using WORDPERFECT 5.1 (Wordperfect Corporation). The text was type set in the Times Roman font and printed using a Hewlett Packard, HP Laserjet Series II, Laser Printer.

PRESENTATION OF THE DATA

Many of the figures have been presented with the variable 'day of the year' (*i.e.* time) along the abscissa. Day of the year was used in preference to 'Julian Day' for the reasons given by Wilimovsky (1990). The first day of any calendar month is given along the abscissa. During each season, the periods of study were carried out over two consecutive calendar years, and therefore the first of January of the second year has been given as day 366 (*i.e.* days in the 'second' year of any season have been calculated as 'day of that year, plus 365'). Figure 2.7

gives this explanation rather more pictorially, and indicates the seasonal periods of the Southern Hemisphere. Reference is made to this figure throughout the remaining chapters.

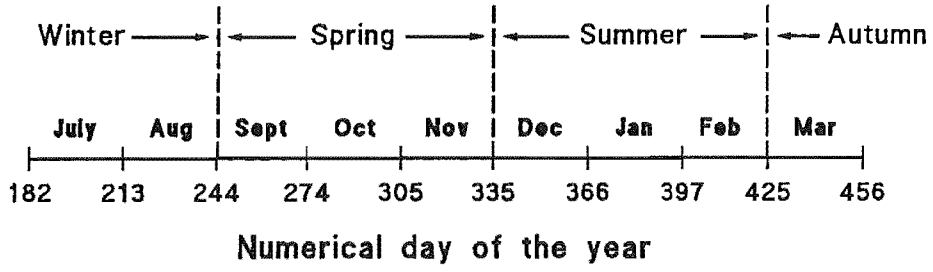
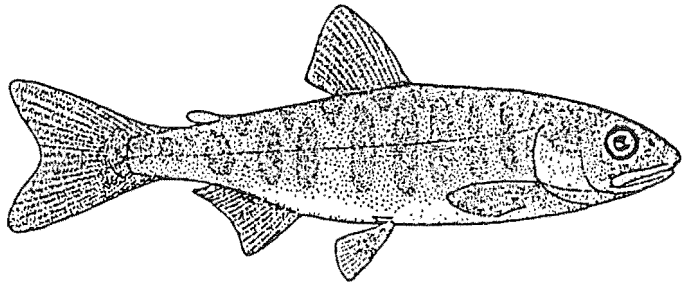


Figure 2.7 Numerical representation of the first day of each month, presented on a 'day of the year' basis. Day of the year has been presented as the variable along the abscissa in many of the figures in the following chapters. Calendar months have been given here for comparison. Because experiments in each season were carried out over two consecutive calendar years, day of the year for months in the 'second' year were calculated as 'day of that year, plus 365'. Approximate austral seasonality has also been given.

CHAPTER THREE

EXERCISE TRAINING CAN RETARD THE GROWTH RATE OF UNDERYEARLING CHINOOK (*Oncorhynchus tshawytscha* Walbaum), AND SOCKEYE (*O. nerka* Walbaum) SALMON



CHAPTER THREE

EXERCISE TRAINING CAN RETARD THE GROWTH RATE OF UNDERYEARLING CHINOOK (*Oncorhynchus tshawytscha* Walbaum), AND SOCKEYE (*O. nerka* Walbaum) SALMON

INTRODUCTION

Salmonids have been an important food source for centuries, particularly among the Innuit and Red Indian tribes of North America and the Arctic, and the native people of Europe. Lore, legend and myth abounds in the many thousands of publications on these fish. The scientific literature has become inundated with papers on salmonids biology. In conjunction with the development of the salmon farming industry, much of the research has focused on optimising production of farmed salmonids.

The rudimentary knowledge for artificial propagation of salmonids has been known for centuries; the first such record may be accredited to a German Lieutenant Jacobi, in a Hanoverian journal in 1763 (later translated into French by Georges Louis Buffon in 1783; given in Netboy, 1974: page 334). In 1872, the United States Fish Commission authorised and established the first hatchery for Pacific salmon in northern California. The hatchery, Baird Station, was constructed by Livingston Stone, on the McCloud River, a high country tributary of the great Sacramento River, known to be a prolific river for sea-run chinook. The chinook that run in the east coast rivers of the South Island of New Zealand were established from eggs and milt taken from fish caught in racks from the McCloud itself, and Battle Creek, another tributary 24 km downstream (US Fisheries commission Reports, 1900-1908).

Chinook are a prized fish for many anglers, due to their sheer size (the largest salmon) and the good fight they offer when hooked. Their growth and development has been extensively studied, both in North America and New Zealand (Rutter, 1904; Gilbert, 1913; Rich, 1920; Clark, 1929; Stokell, 1962; Parrot, 1971; Flain, 1972a,b, 1981, 1982; Healey, 1980a,b, 1982, 1983, 1991; Unwin, 1981, 1986; Healey and Heard, 1984; Taylor and Larkin, 1986; Healey and Groot, 1987; Taylor, 1988a,b, 1989, 1990a,b,c, 1991; Franklin, 1989). They are subject to many farming and stock enhancement programmes within their natural and 'extended' range.

In New Zealand, one company, Peacock Springs Salmon, grow chinook to 2-3 kg, wholly in freshwater - possibly the only such venture in the world. Many other fish farms in New Zealand on-grow chinook to market size in sea cages, in bays and inlets of both the South Island and Stewart Island. The scale of such commercial operations pale in comparison to those in North America.

Much of the research on salmonids aims to enhance growth and growth rate of these fish. The goal of such research is to achieve as much weight of wet flesh per unit weight of feed consumed, *i.e.*, effecting greater efficiency of growth. The methods by which growth is evaluated are varied and have become increasingly 'hi-tech'. Morphological measurements of

the body are the most common. Analysis of the circuli (ring) pattern laid down in the scales and the otoliths were first used at the turn of the century (Gilbert, 1913). In the recent past, the rate of radiolabelled amino acid uptake by scales (reviewed by Adelman, 1987) and tissues (reviewed by Houlihan, 1991) have been correlated to somatic growth rate.

It is conceivable that the greatest advances in promoting growth have been made with improvements in nutrition - particularly the development of 'Oregon moist' and 'Abernathy dry' pelleted feeds (and subsequent analogues) and fish husbandry. Pelleted feed is readily acceptable and digestible by the fishes. Additionally it can be rolled, chopped and crushed into known particle sizes, accurately tailored to the gape size of the fish. This matching of feed particle size to fish size is very important in maintaining a high growth rate (Thorpe and Wankowski, 1979; Wankowski, 1979; Wankowski and Thorpe, 1979b).

Research has shown that nutritional requirements of fry, parr, smolt, post-smolt, and sub-adult fish differ throughout growth and development. These changes are met through alteration of feed mixture recipe at each pellet size. Pellets are further advantageous; antibiotics and other compounds may be added during production and thereby orally administered, without fish handling. Whilst pelleted feed is in many ways advantageous, it is expensive, and the outlay must be recovered at the point of sale. *Ad Libitum* feeding from automatic feed hoppers, does promote fast growth, but is extremely wasteful, particularly in the light of new research indicating that salmon appetite is diurnal. The work suggested that wastage could be limited if feed delivery was tailored to daily rhythms of hunger (Kadri *et al.*, 1991). Furthermore, appetite in salmonids is seasonal, and feeding regimes should be matched accordingly (Metcalfe *et al.*, 1986).

Alteration of photoperiod, the natural oscillation of day (light) and night (dark), has been investigated as a tool for enhancing growth. Salmon are visual predators, feeding only during the hours of light, most voraciously at dawn and dusk (Thorpe *et al.*, 1990b). Obviously therefore, if the hours of daylight are extended (beyond natural), feed may be presented over a longer period. Fish have 'biological clocks' - natural endogenous rhythms that regulate processes of development via neural/hormonal control. These endogenous rhythms are kept in phase with the environment, through daily entrainment by the natural photoperiod (Eriksson and Lundqvist, 1982). Light from the sun and the moon are thought to be involved (Farbridge and Leatherland, 1987a,b,c). Therefore, such rhythms are likely to be affected by any given alteration of photoperiod. Manipulation of photoperiod has many applications; pregnancy rates in ewes have been enhanced following melatonin administration (Robinson *et al.*, 1991; 1992a,b). The peptide hormone melatonin is released from the pineal gland in the absence of light, *i.e.* shorter days and ewes are triggered into reproductive condition in the autumn (Robinson *et al.*, 1992a). Salmonids of all ages have been variously subjected to photoperiod manipulations, with some studies showing enhanced growth rates (recently reviewed by Hoar, 1988; Zaugg *et al.*, 1986).

Another alteration of environment as a possible means of growth enhancement has been to increase ambient water temperature (reviewed by Brett, 1979; Brett and Groves, 1979; Elliot,

1979; Jobling, 1983a). As with alterations of photoperiod, temperature changes can have positive and negative effects on 'normal' growth and development. Biological systems are sensitive to temperature, and have functional optima. Salmonids are rather stenothermal (the viable temperature range decreases with age), and become stressed and increasingly susceptible to disease at temperatures close to their lethal maxima. Low water temperatures reduce growth rates. Discrete stocks and populations are likely to be adapted to their particular environment (Ricker, 1972). Therefore, whilst slight increases in temperature may raise somatic growth *per se*, other developmental processes may be enhanced, retarded, or even inhibited (Hoar, 1988; Soivio *et al.*, 1988). Recently, Berg and co-authors have demonstrated that Atlantic salmon kept at a constant temperature did not grow as fast as salmon maintained in a system where the water was warmed and cooled on a daily cycle (Berg *et al.*, 1990). In their study, the daily thermal/time units were equivalent for both groups.

Experiments using various hormones to stimulate growth have yielded positive results (comprehensively reviewed by Donaldson and co-authors in 1979; and more recently by Weatherley and Gill in 1987). Almost all hormones so far administered have produced a measurable somatic effect, particularly growth hormone (somatotropin), its analogues, and the steroid hormones. Although the early hormone work was achieved using limited hormone extracts from other animals (of bovine, ovine and porcine origin), advances in recombinant DNA technology could possibly allow for expansion of growth promoting, hormone mediated treatments, particularly with transgenic fish (Agellon *et al.*, 1988a,b; Renard *et al.*, 1989).

Hormone administration, and alteration of the DNA genome, are currently major ethical issues, presenting to 'Society' serious negative connotations and repercussions, whether or not they are warranted. The greatest, single factor for the increase in agricultural livestock and salmonid production, is attributable to improvements in husbandry and nutrition (Raymond and Neimann-Sørensen, 1989). Unlike livestock, salmonids are marketed as luxury, rather than staple commodities. The growth of commercial salmonid farming has occurred due to the opening and expansion of a market niche (as an offshoot of conservation measures to 'protect' wild stocks), rather than strong market demand, due to post-war shortage. Currently, as a result of over production, there is a world glut of farmed salmonid flesh. As with the dairy industry for instance, salmon farmers are more likely to survive by reducing their costs of production, rather than producing more and larger fish (Raymond and Neimann-Sørensen, 1989). Hormone use for growth enhancement in salmonid farming is not widespread at the present time, and is banned in several countries.

The effect of various forms of exercise and exercise training (low intensity, long duration swimming) on growth has been investigated in many teleosts. The prospect that exercise training may produce a larger carcass with more muscle, and the widespread belief that 'sluggish' farmed fish are of inferior quality to wild fish, has led to much research involving exercise as the growth promoter (reviewed by Davison, 1989). Enforced swimming has been achieved by various methods. However exercise training studies on fish are not as strictly controlled as those involving mammals. Consequently, meaningful comparison between studies

is often limited. Exercise training in mammals produces many measurable alterations of physiology, which are commonly and collectively thought to reflect improvement in 'general' health and physical condition. Mammalian physiology is often termed 'plastic' with respect to its ability to respond to experimentation. The physiology of the lower vertebrates, such as teleosts, is far less malleable, although responses to training have been noted. Initial studies of exercise training on teleost growth (Greer-Walker, 1971; Greer-Walker and Pull, 1973) indicated that fish muscle fibres responded to low intensity swimming with hypertrophy (increase in muscle fibre diameter) and hyperplasia (increase in muscle fibre number) of the lateralis muscle.

The interest generated by these findings precipitated further exercise training work, particularly among the salmonids. Davison and Goldspink (1977) reported that the growth of trained brown trout (*Salmo trutta*) was better than control fish, when they were subjected to 28 days of continuous exercise at 1-1.5 body lengths per second ($bl.s^{-1}$). Additionally they found that growth of trout trained at 3.0 or 4.5 $bl.s^{-1}$ was retarded compared to the controls. The enhanced growth of the 1-1.5 $bl.s^{-1}$ group was attributed to hypertrophy of all three muscle fibre types. The exercise group also demonstrated improved food conversion efficiency (Davison and Goldspink, 1977).

Enhancement of growth rate with exercise training over prolonged periods (30 days or longer) has been subsequently reported in the rainbow trout, *Oncorhynchus mykiss* (Greer-Walker and Emerson, 1978; Nahhas *et al.*, 1982a; Houlihan and Laurent, 1987; Farrell *et al.*, 1990); Atlantic salmon, *Salmo salar* (Kuipers, 1982; Totland *et al.*, 1987); lake charr, *Salvelinus namaycush* (Leon, 1986); brook charr, *Salvelinus fontinalis* (East and Magnan, 1987); and Arctic charr, *Salvelinus alpinus* (Christiansen *et al.*, 1989; Christiansen and Jobling, 1990). Pacific salmon are largely lacking from this comprehensive list of exercise training research. Additionally, virtually all of the studies above (excepting the work on Arctic charr) have been carried out on fish that were yearlings or older. It was decided therefore to undertake growth and exercise training studies on underyearling chinook salmon.

Three differing exercise training regimes were imposed on sibling groups of chinook during their first nine months of life (season 1, October 1989 - December 1989; season 2, September 1990 - March 1991; season 3, June 1991 - December 1991). Growth and growth rates and a number of morphological and physiological parameters were compared between the trained and untrained fish. In addition, the data on hatchery chinook, 'wild' (*i.e.* naturally spawned) chinook, and hatchery sockeye salmon (*Oncorhynchus nerka*) were subjected to training experiments.

MATERIALS AND METHODS

Fish stocks and study sites

Two study sites were used in this study. The work during season 1 was performed at a commercial salmon farm. The Aquarium Room of the Zoology Department (hereafter the

Department), Canterbury University - the second study site - was the location for all the subsequent work of seasons 2 and 3.

All fish used in the training experiments were underyearling (0+) salmon. The hatchery chinook (and sockeye) originated from the Ministry of Agriculture and Fisheries' (MAF) Glenariffe Hatchery. Wild chinook were caught in the fry trap, Glenariffe Stream. All linear and weight measurements were determined on lightly anaesthetised fish. In season 2, some fish were individually marked with Alcian Blue dye. In season 3, marking was facilitated with a cold branding technique (Herbinger *et al.*, 1990; *see* CHAPTER 2, Figure 2.2 and surrounding text). Measurement of yearling, and two year old, freshwater reared chinook was possible with grateful thanks to Mr T Crowe of Peacock Springs Salmon Farm.

Season 1: - long term (85 day), continuous swimming

All experimental trials were undertaken within a specially constructed, four lane fish race, positioned in one of the salmon farm's raceways (*see* CHAPTER 2, Figure 2.3 and surrounding text). The design of the race was similar to that of Nahhas and associates (1982a). The 'swimming compartment' of each lane was identical. On 6 October 1989, 40 fish were measured into each lane. After a 48 hour recovery period, and during the course of the following 48 hours, the water flow into the 'exercise training' lanes was increased and adjusted so that water speeds of 2.5, 1.7, and 1.0 body lengths per second ($bl.s^{-1}$), relative to fork length, were attained. Water velocity through the fourth lane was maintained at less than 0.25 $bl.s^{-1}$, the fish in this lane were the 'still water' controls (untrained fish). The water speed in each lane could not be increased during the experiment.

A large wooden board was placed over the four lanes to afford the salmon cover. Fish were reared under natural photoperiod and fed every 15 minutes during the hours of daylight from automatic feed hoppers. On 25 October, 10 and 29 December 1989, the fish were remeasured. Ten fish were killed by overanaesthesia on each such sampling occasion, and dissected.

Season 2: - short term (ten day), continuous swimming

Exercise training was effected with a 175 litre oval racetrack flume (*see* CHAPTER 2, Figure 2.4 and surrounding text). Actual water velocity was increased during the seasons, ensuring that the relative swimming speed was 1-1.5 $bl.s^{-1}$ (initial fork length) for all trials. Untrained fish were reared in still water, within a glass tank. Compressed air was gently bubbled through air stones ensuring good water aeration in the flume and tank.

Fortnightly training trials were performed on sibling groups of chinook salmon from 2 November 1990 to 15 March 1991. For each trial, groups of six fish were measured and placed in the glass tank or flume. Fish were allowed to recover from transportation and handling stresses for 60-70 hours prior to the start of the continuous training period. All fish were fed to satiation by hand at least five times daily.

Season 3: - short term (ten day), short duration swimming

The design of the third season's training regime was identical to that imposed in the second, except that the training period was reduced to eight hours a day (9 a.m. to 5 p.m.). Untrained fish were reared as for season 2. Trials were performed fortnightly from 28 June to 24 October 1992. Hatchery sockeye salmon and 'wild' chinook (six fish per group) were subjected to exercise training trials; growth was compared to untrained fish in still water tanks.

Fish sampling and dissection

Measurements of trained and untrained fish were made at the beginning (initial) and end (final) of each trial. In season 3, standard length, and length of the caudal peduncle were additionally recorded (*see* CHAPTER 2, Figure 2.1 and surrounding text). Fish were killed by overanaesthesia. Once measured, the tail was severed and blood collected into ammonium heparinised micro capillaries, which were then plugged and centrifuged at 5000 *g* for six minutes. Blood haematocrit was measured. Plasma in the micro capillaries was transferred to plastic vials and snap frozen in liquid nitrogen. A sample of white muscle was dissected from the tail stump and weighed. The viscera and heart were similarly dissected and weighed. These samples were then dried to constant weight. The gills were removed, rinsed in homogenising buffer (Johnson *et al.*, 1977), placed into plastic vials, and snap frozen in liquid nitrogen. All samples were stored at −80 °C, until further analysis.

Physiological analysis

Blood plasma was analysed for sodium and chloride ion concentration ($mmol.l^{-1}$) and osmolality ($mOsm.kg^{-1}$). Sodium concentration was analysed in a Varian Techtron 1200 Absorption Spectrophotometer. Chloride concentration was determined using a Radiometer CMT 10 chloride titrator. A Wescor Inc. 5100C vapour pressure osmometer was used to determine plasma osmolality. Plasma was pooled, as necessary, prior to analysis, to facilitate that all three determinations were measured for each experimental group. Activity of the enzyme Na^+-K^+-ATPase was measured in the gill samples, using previously described methods (Johnson *et al.*, 1977; Langdon *et al.*, 1984; Franklin, 1989).

Calculation of growth and morphological indices

Fulton's condition factor (CF), $100 \times W/L^3$ (where W is the gram weight, and L is the fork length in centimetres), was calculated for each fish at every measurement. A carcass condition factor (CCF) was also calculated for each fish upon dissection ($CCF = 100 \times W_c/L^3$ (where W_c is the gram weight of the carcass (*i.e.* body weight−viscera weight), and L is the fork length in centimetres). Growth rates were calculated for both untrained and trained fish as linear growth rate (LGR), using $[L_f - L_i]/t$ (where L_f and L_i are the final and initial fork lengths (mm) respectively, and t is the duration of the growth period in days); and specific growth rate (SGR), using $[[\ln(W_f) - \ln(W_i)]/t] \times 100$ (where $\ln(W_f)$ and $\ln(W_i)$ are the natural logarithms of the final and initial wet weights (g) respectively, and t is the duration of the growth period in days).

Indices of standard length and length of the caudal peduncle were calculated as a proportion of fork length. Cardiac and visceral indices were calculated as a proportion of total weight. Percentage water content of the white muscle, heart and viscera were also calculated. All data were entered into spreadsheet software for calculations and statistical analysis.

Statistical analysis

Results are presented as individual measurements, the mean ±standard error, or ±95% confidence limits (n=6 unless otherwise indicated). The male symbol, ♂, indicates that in that trial, one of the fish was found to be precociously maturing. Data concerning the precocious fish were excluded from the analyses presented in this chapter, and therefore n=5 for the data from that group (Chapter 7 details the changes associated with this, and one other maturing chinook that was recovered from a seawater growth experiment). Homogeneity of the initial group variances were checked using an F_{max}-test. Statistical significance was assessed using one way analysis of variance (one way ANOVA), and the Student's unpaired t-test. Significance was recorded when $p \leq 0.05$.

RESULTS

Fish behaviour

Exercise fish readily took to all three swimming regimes. In season 1, initial reluctance to swim in the fastest lane of the racetrack (2.5 bl.s^{-1}) was not noted after three days of training, and therefore it was not necessary to replace 'non-swimming' fish as has been the case in other studies (Davison and Goldspink, 1977). Fish in all four lanes oriented into the current, and positioned themselves throughout the length of each lane, underneath the wooden cover. Fish were observed to strike predominantly more at feed as it passed by them, rather than at the surface - probably in order that they held their position in the lane. Aggression and competition between fish were noted, but fin damage was not observed on sampling occasions, indicating that overall, competition was not injurious. It was not possible to determine whether the fish swam in 'school' formations due to the wooden cover.

In seasons 2 and 3, the exercise trained fish oriented into the current and generally held individual positions in the swimming compartment. Schooling behaviour was not observed at any time. Aggressive behaviour between fish was noted, being limited to chasing and darts. Actual nips or bites were not observed (although these may have occurred). Fin damage was not observed in either the trained or untrained fish. Feed was distributed by hand throughout the chamber/tank in order that each fish was afforded equal opportunity to feed. While fish occasionally took feed from the surface, striking at feed as it drifted past in the water column was the norm. Fish were rarely observed to take feed from the bottom of the flume.

Untrained fish in the still water tanks were generally quiescent, resting on their pectoral and caudal fins at the bottom of the tank. An analysis of their activity pattern was not

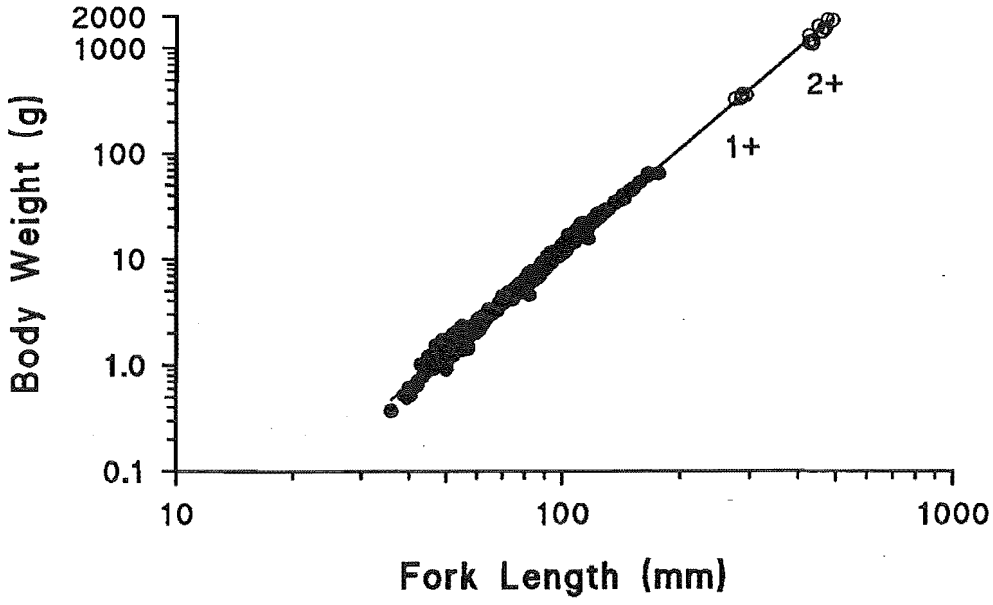


Figure 3.1 Regression of weight on fork length for freshwater reared chinook. Both axes have logarithmic scales. Data represent all values for untrained fish from each season (underyearlings: solid circles), and values from freshwater reared yearling and two year old chinook (1+ and 2+ respectively: open circles).

undertaken. Although occasional chases, darts and attacks were noted between fish, dominance hierarchies were not obvious, excepting that subordinate fish would often 'hide' under the outflow pipe. Attempts to determine dominance were made with the individually branded fish, by recording the first fish to feed at each feeding bout. However, no obvious frequency pattern of first feeding by each fish was noted. Tank size, low stocking densities, and *ad libitum* rations probably reduced competition at feeding periods. Feed was predominantly taken at the surface by the untrained fish, often in a 'frenzy'. Whilst feed was also taken from mid water, bottom feeding was rarely observed.

Growth

Growth in terms of length and weight, and the associated change of fish condition was followed over the three seasons. Data for untrained fish of each year and data from the sample measurements of Peacock Springs farm fish were pooled and plotted in Figure 3.1 (logarithmic axes). The relationship is described by the curve $\log Y = 3.17(\log X) - 5.27$.

The results of season 1 are presented in Figure 3.2. There was no discernable difference ($p > 0.05$, one way ANOVA) in any parameter between the three trained and untrained groups. The ten farm fish measured on 10 and 29 December 1989 were significantly larger and in 'better' condition ($p < 0.0001$, one way ANOVA) than the 'experimental' (trained and untrained) fish held in the four lane race.

Figure 3.3 illustrates the changes of condition factor (CF), carcass condition factor (CCF), linear (LGR) and specific growth rate (SGR) with time, for untrained and trained fish

from season 2 and season 3. CF of untrained fish increased (almost doubling) over the course of the investigation from 0.67±0.03 (mean±SE) in 'zip-up' fry, reaching a plateau of approximately 1.33±0.03 over the summer months in 'post-smolts' (December onwards). CF of untrained fish was significantly greater than trained fish, where comparisons were possible ($p<0.0001$, one way ANOVA). CCF was also significantly higher ($p<0.001$, one way ANOVA) in the untrained fish in all the trials.

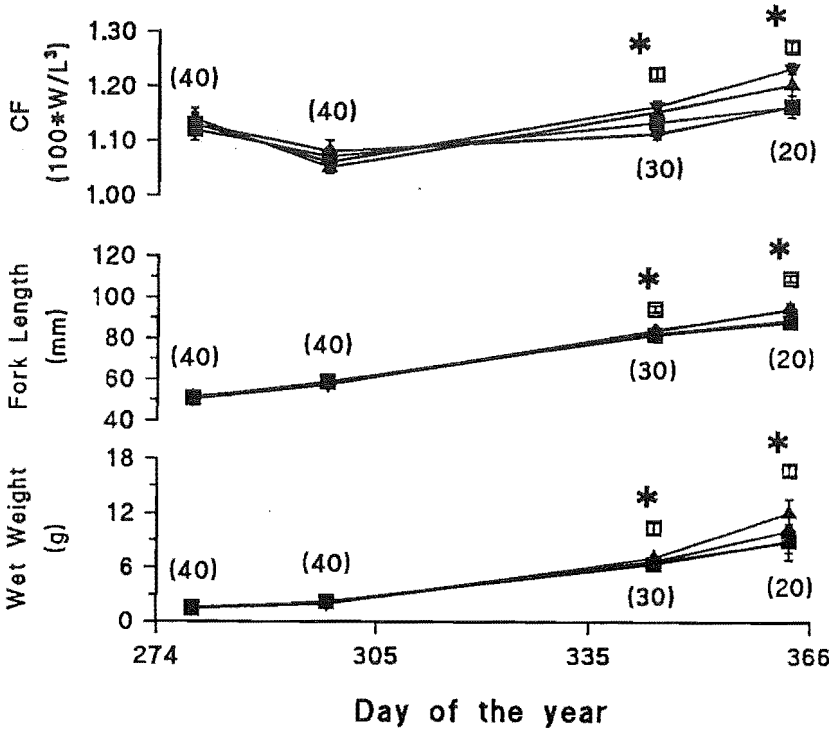


Figure 3.2 Changes of condition factor (CF), length and weight of season 1 chinook with time (day of the year). Abscissa numbers represent the first day of the months of the year, *i.e.* 274 is the first of October (*see* CHAPTER 2, Figure 2.7). Each point represents the mean ±standard error (variable *n*, the number of fish measured on each occasion is given in parentheses). Solid symbols represent the 'experimental' (one untrained and three trained groups) fish. Open squares represent data from sibling 'farm' fish ($n=10$) taken from a farm raceway. Significant differences between the 'experimental' and 'farm' fish is shown by asterisks (*).

Untrained fish had a significantly faster LGR than trained fish ($p<0.0001$, one way ANOVA). The graph of LGR for untrained fish described a 'bell-shaped' curve with time. An initial low rate (0.38±0.05 mm.day-1) in July increased to a maximum of 0.84±0.03 mm.day^{-1} (during spring), and decreased thereafter (Figure 3.3). LGR of untrained fish averaged 0.48, 0.60, and 0.54 mm.day^{-1} for season 1, 2 and 3 respectively. Comparison of SGR between trained and untrained fish reveals that training also had a definite inhibitory effect of growth in terms of weight gain ($p<0.001$, one way ANOVA). SGR was high but not maximal in the youngest fish ('zip-up' fry). SGR decreased with time from a maximum of 5.06±0.41% to 0.93±0.17% body weight.day^{-1} (Figure 3.3).

The two points (untrained fish) marked by arrows in Figure 3.3, were obtained from fish

that were fed the wrong feed size (too small, as determined from the manufacturer's recommended feed size charts). As a result of this feeding regime, the growth rates recorded by these fish were low compared to those calculated for similar sized fish fed the correct feed particle size.

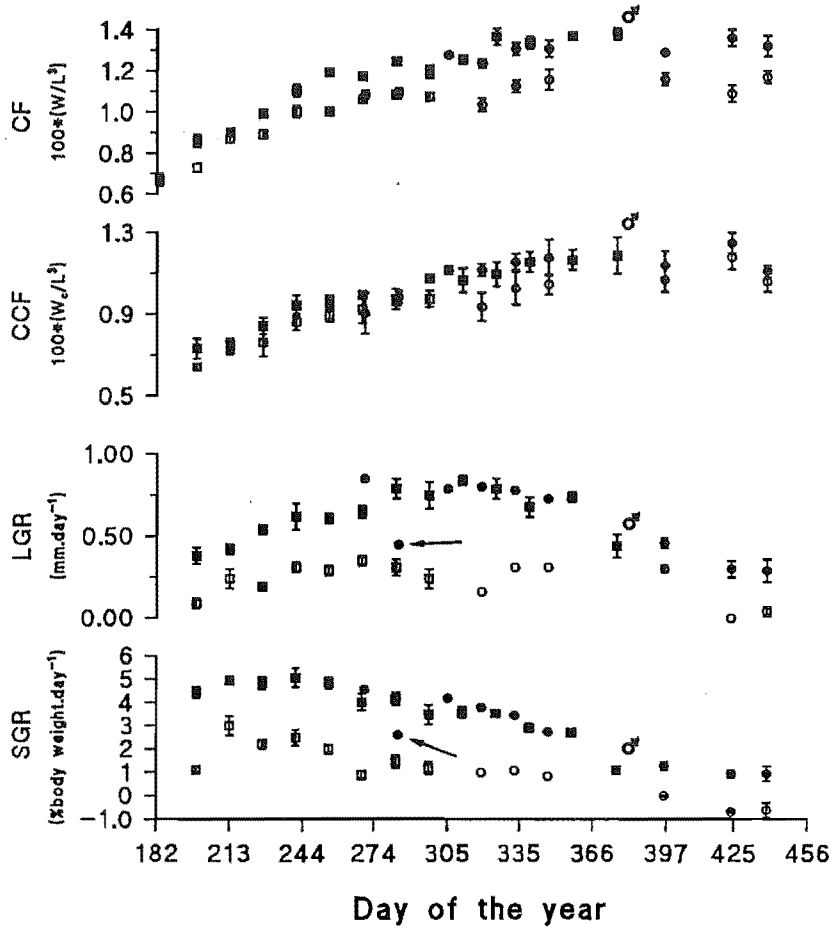


Figure 3.3 Changes in condition factor (CF), carcass condition factor (CCF), linear growth rate (LGR), and specific growth rate (SGR) of freshwater reared chinook salmon with time (day of the year). Each point represents the mean ±standard error (n=6, except the points marked by the male symbol, ♂, where n=5). Circles represent data from season 2, and squares the data for season 3. Open and solid symbols are exercise trained and untrained groups respectively. The data points indicated by arrows are explained in the text. 'Day of the year' is used as the variable on the abscissa in preference to Julian Day (Wilimovsky, 1990). Because growing seasons over-ran the end of one calendar year and the beginning of the next, dates for sampling occasions in January, February and March (in the following year of that growing season) are given as day of that year plus 365.

The effect of exercise training on the growth of wild chinook is shown in Table 3.1. Growth rate data for similar sized hatchery chinook are included for comparison, and show that wild fish did not grow as fast as their hatchery counterparts ($p<0.001$, one way ANOVA). As

with the hatchery chinook, the training regime imposed (8 hours.day^{-1}) significantly slowed ($p<0.005$, one way ANOVA) both the linear and specific growth rates of the exercised fish.

Growth data for the exercise training experiment with sockeye salmon are presented in Table 3.2. As with Table 3.1, growth rate data for similar sized chinook salmon have been added for comparison. Sockeye did not grow as fast as chinook salmon of an equivalent size ($p<0.005$, one way ANOVA). Exercise training did not affect the growth rate of sockeye salmon (SGR, $p=0.09$; LGR, $p=0.22$).

Table 3.1 Specific and linear growth rates of exercise trained and untrained wild chinook (growth rates of similar sized, untrained hatchery chinook are added for comparison). Data are presented as mean ±95% confidence limits ($n=6$). Statistical significance ($p\leq0.05$, one way ANOVA) is indicated by asterisks (*) between wild fish, and by single daggers (†, $p\leq0.001$) between wild and hatchery (untrained) chinook.

	WILD CHINOOK		HATCHERY CHINOOK
	Untrained	Exercise	Untrained fish (@7)
SGR (%wt.day^{-1})	2.29±0.66 *	1.02±0.49	4.15±0.66 †
LGR (mm.day^{-1})	0.32±0.15 *	0.12±0.05	0.79±0.15 †

Table 3.2 Specific and linear growth rates of exercise trained and untrained sockeye salmon (growth rates of similar sized hatchery chinook are added for comparison). Data are presented as mean ±95% confidence limits ($n=6$). Statistical significance (one way ANOVA) is indicated by daggers, (†, $p\leq0.005$) between sockeye and hatchery (untrained) chinook.

	SOCKEYE		CHINOOK
	Untrained	Exercise	Untrained fish (@9)
SGR (%wt.day^{-1})	1.56±0.31	1.14±0.32	3.56±0.59 †
LGR (mm.day^{-1})	0.39±0.09	0.32±0.09	0.84±0.07 †

Standard length and length of the caudal peduncle increased with fork length as the fish grew (data not shown). Both parameters were converted to 'percentage of fork length' indices, and are plotted (mean ±95% confidence limits) against day of the year in Figure 3.4 (data from season 3 only). The caudal peduncle index was initially constant. It increased between the third and seventh sampling occasions (mid August - mid October), and then returned to the initial level thereafter. The index of standard length was initially constant, increasing between the fifth and sixth sampling date (late September) to a new constant level. A one way analysis of variance between trained and untrained fish demonstrated that exercise training (8 h.day^{-1}) had no effect on either the length of the caudal peduncle ($p=0.46$), or the standard length ($p=0.14$).

Viscera weight and heart weight of untrained fish increased exponentially with time, and scaled with body weight, as shown in Figure 3.5 (pooled data from season 2 and 3). Data from

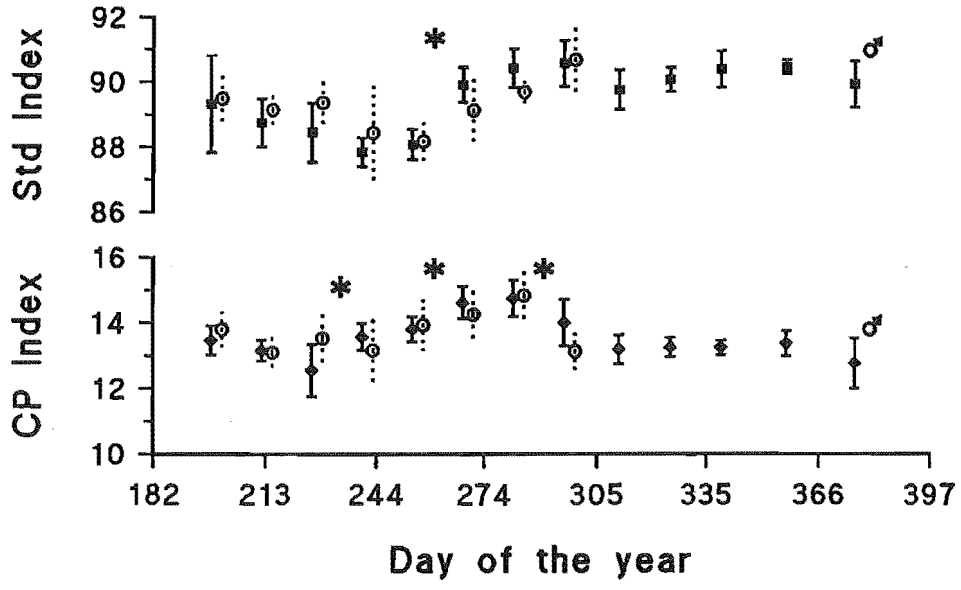


Figure 3.4 Changes in the indices of standard length and length of the caudal peduncle with time (day of the year). Data are presented as mean ±95% confidence limits (n=6, except the points marked by the male symbol, ♂, where n=5). Solid and open symbols represent untrained and trained groups respectively. The data points have been off-set with respect to sample date to permit comparison between groups. Statistical significance ($p<0.05$, unpaired t-test) between sample means of growth fish is given by asterisks (*).

yearling (1+) and two-year old (2+) freshwater reared chinook (Peacock Springs Salmon Farm) have been added for comparison. The regression lines for the heart and viscera data describe the relationships $\log Y=0.924(\log X)+0.165$ ($r^2=0.994$), and $\log Y=0.898(\log X)-0.837$ ($r^2=0.990$), respectively. Heart weight accounted for 0.15±0.02% (mean±95%CL, n=6) of total body weight in 'zip-up' fry, and decreased to 0.11±0.01% in underyearling post-smolts, 0.10±0.00% in yearling, and 0.09±0.01% in two year old salmon. Visceral weight was 14.45±1.97% of total body weight in 'zip-up' fry, 12.61±0.73% in underyearling post-smolts, 7.67±0.88 in yearling, and 8.00±0.39% in two year old fish. The decrease in relative weight of both tissue types was significant ($p<0.05$, one way ANOVA) between 'zip-up' fry and each older age group. Training had no effect ($p>0.05$, one way ANOVA) on heart weight but significantly reduced viscera weight ($p<0.05$, one way ANOVA). There was no significant difference ($p>0.05$, one way ANOVA) in heart weight between wild and hatchery chinook of the same body weight. However, viscera weight was significantly lighter ($p<0.05$, one way ANOVA) in the trained, wild fish group.

Regression lines describing the relationship between heart weight, and viscera weight *versus* body weight for sockeye salmon are $\log Y=0.834(\log X)+0.320$, and $\log Y=0.935(\log X)-0.792$, respectively (data for sockeye have not been plotted in Figure 3.5). Heart weight represented 0.20±0.01% of body weight in five gram fish, and decreased to 0.12±0.01% in 30 gram fish. The visceral index represented 14.43±1.05% and 13.04±1.40%

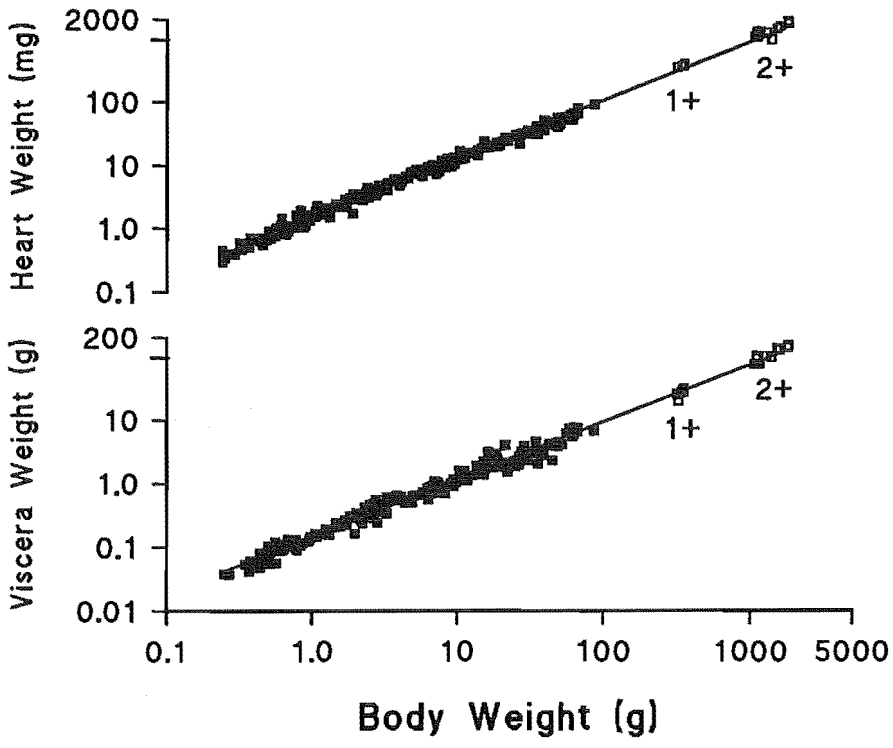


Figure 3.5 Plots of heart and viscera weight against body weight for chinook salmon. Both axes have logarithmic scales. Data points represent individual untrained fish from season 2 and 3 (solid symbols), and Peacock Springs farm fish (1+; yearling, and 2+; two year old fish, open symbols).

over the same size range. Comparison between the species demonstrated that both the heart and the viscera accounted for a larger proportion of total body weight in sockeye ($p<0.05$, one way ANOVA).

Percentage water content of the swimming muscle (white), heart and viscera changed over each season of growth (Figure 3.6, data from season 3 only). Training had no effect (data not shown) on the percentage water content of any tissue type ($p>0.05$, one way ANOVA). The greatest drop in percentage water content with time was found in the viscera; decreasing from $76.95\pm0.62\%$ (mean$\pm$95%CL, $n=6$) in 'zip-up' fry to $64.23\pm1.49\%$ in 'post-smolts'. Water content of the viscera decreased steadily as the fish grew (visible adipose tissue, lining the intestinal tract, increased with fish size).

Heart muscle recorded the consistently highest percentage water content. Initially, heart water content was $79.92\pm0.64\%$. It reached a maximum of $81.46\pm0.32\%$ (sixth sample, late September), and then declined thereafter to $80.46\pm0.39\%$ (early January). Percentage water content of the white muscle decreased from an initial maximum of $81.92\pm0.82\%$ to $78.97\pm0.45\%$ during season 3 ('zip-up' fry to post-smolt). Muscle water content dropped sharply ($p<0.05$, one way ANOVA) on the third sampling occasion of season 3. Water content of the white muscle was also significantly lower ($p<0.05$, one way ANOVA) during the sixth and seventh sampling occasions (late September - early October), at approximately 77%. Tissue

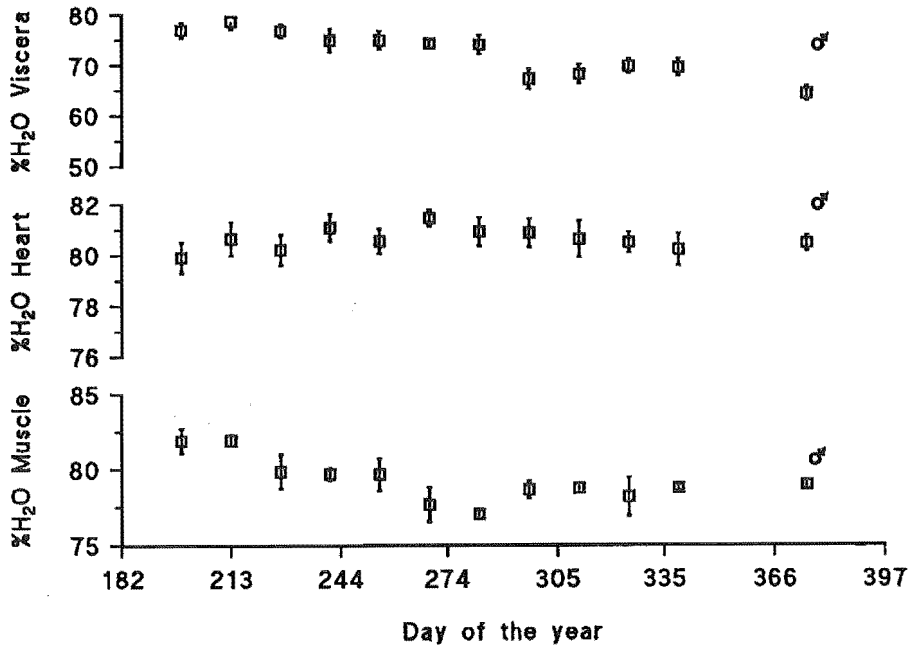


Figure 3.6 Change in percentage water content of untrained, underyearling chinook salmon viscera, heart muscle, and white muscle with time (day of the year). Data points represent the mean ±95% confidence limits (n=6, except the points marked by the male symbol, ♂, where n=5). Data are from untrained fish of season 3 only.

samples taken from two-year old Peacock Springs farmed salmon (freshwater reared) indicated that percentage water content of white muscle, heart and viscera decrease to 75.20±0.44%, 77.48±1.38%, and 60.27±7.78%, respectively.

Figures 3.7 to 3.10 illustrate changes of five physiological parameters (gill Na^+-K^+-ATPase activity [μmol P_i.mg protein^{-1}.h^{-1}], blood haematocrit [percent red cell volume], plasma osmolality [mOsm.kg^{-1}], plasma sodium concentration [mmol.l^{-1}], and plasma chloride concentration [mmol.l^{-1}]) that were measured during season 2 and 3. Figures 3.7 and 3.8 refer to season 2; Figures 3.9 and 3.10 to season 3. Figure 3.7 presents data from untrained fish over the entire season (nine trials), and Figure 3.8 contrasts data from trained and untrained fish, over the six trials where comparisons were possible. Similarly, Figure 3.9 presents all the untrained fish data for season 3 (13 trials), and in Figure 3.10, results for trained and untrained fish are compared (eight trials).

Comparison between seasons (Figures 3.7 and 3.9) show that each parameter varied a great deal over the periods of investigation. The untrained, gill Na^+-K^+-ATPase activity data from both seasons illustrate that enzyme activity increases with fish growth from the 'zip-up' fry to 'smolt' stages of development. Highest levels of enzyme activity were recorded during the natural period of parr-smolt transformation for New Zealand chinook (October to late December) and correlated strongly with fish survival in seawater challenge tests (*see* CHAPTER 5, Figures 5.1, 5.2 and 5.3). Enzyme activity decreased during the summer months

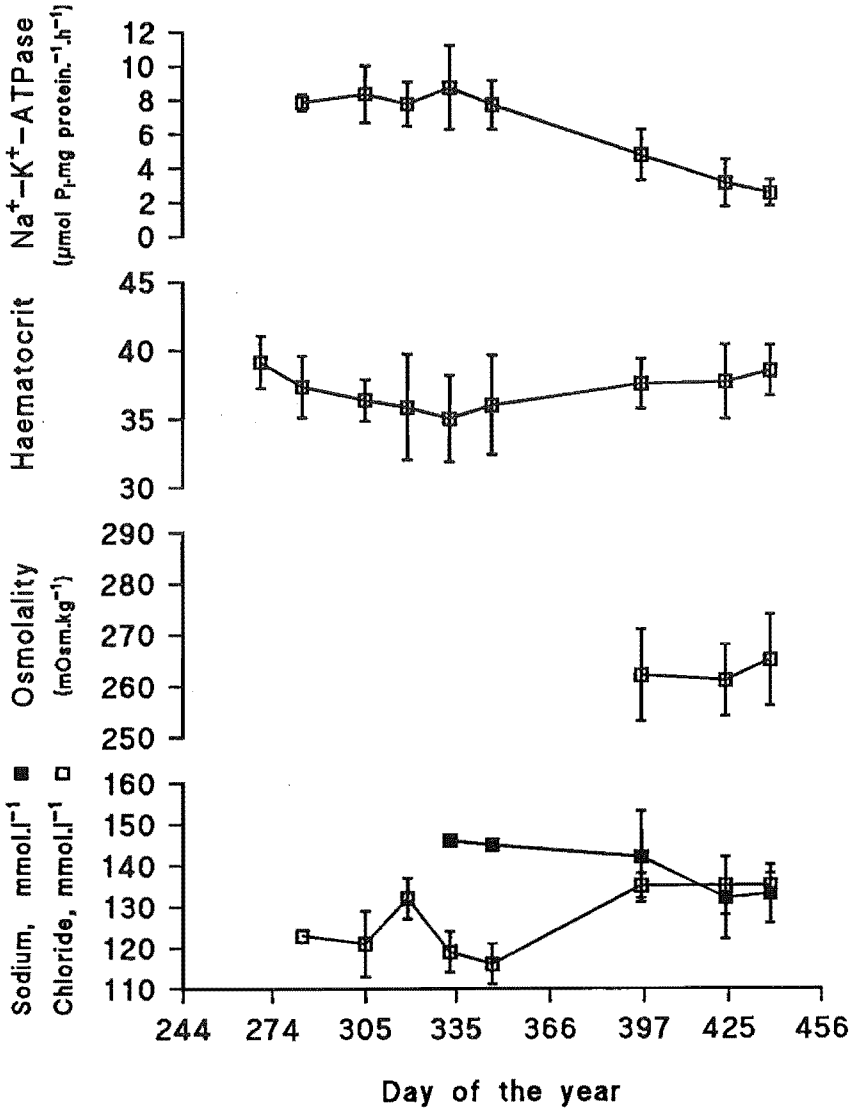


Figure 3.7 Variation in gill Na^+-K^+-ATPase activity, blood haematocrit, plasma osmolality, and plasma concentrations of sodium and chloride in untrained fish from season 2, with time (day of the year). Data points with error bars represent the mean ±95% confidence limits (n=6); data points without error bars indicate that the plasma of the six individual fish was pooled prior to analysis.

(January through March), although this is only clearly evident during the last three trials of season 2 (Figure 3.7).

Although trained fish did exhibit the same general trend in enzyme activity as the untrained fish, exercise training clearly had a negative effect ($p<0.0001$, one way ANOVA) on gill Na^+-K^+-ATPase activity (Figures 3.8 and 3.10). Training caused a reduction in enzyme activity in each trial, and was most marked during the period when activity in untrained fish was maximal (during the period of parr-smolt transformation).

The large error bars (mean±95%CL, n=6) associated with the haematocrit data indicate that blood haematocrit was exceedingly variable between individual fish, in both untrained and

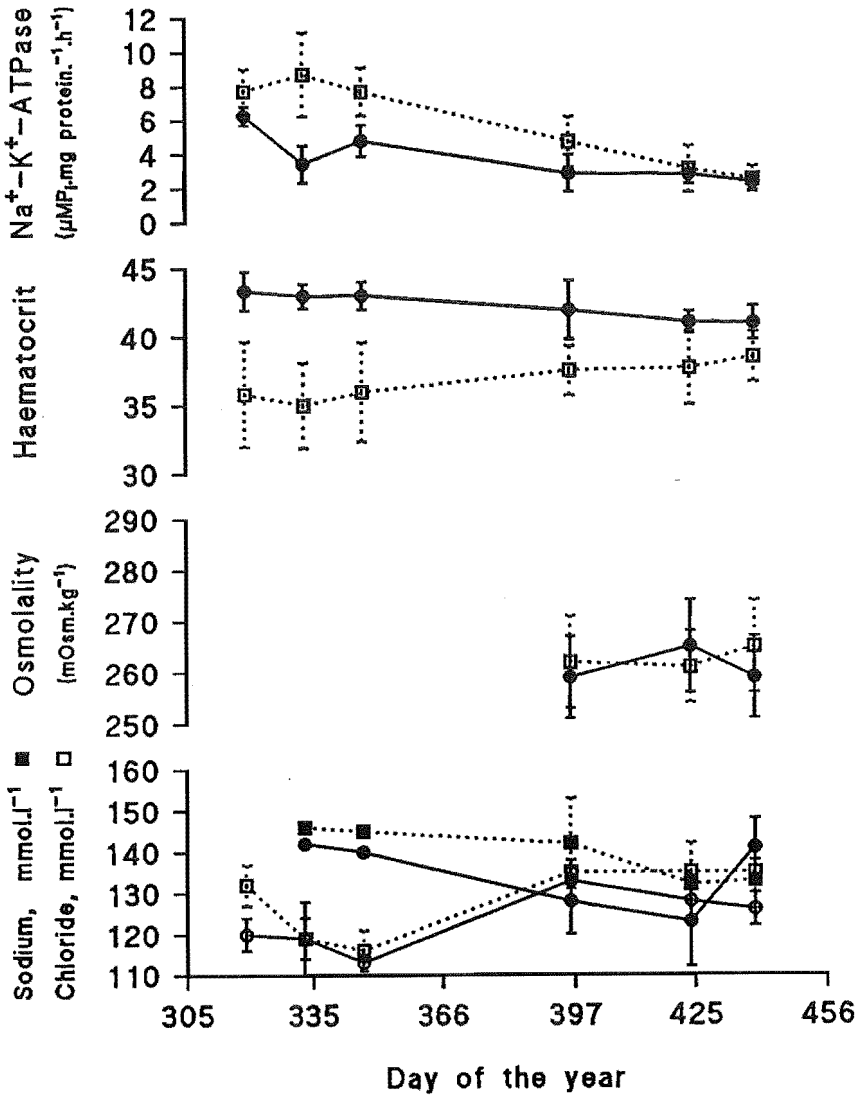


Figure 3.8 Comparison of gill Na^+-K^+-ATPase activity, blood haematocrit, plasma osmolality, and plasma concentrations of sodium and chloride in untrained fish (open symbols, dotted lines) and exercise trained fish (solid symbols, solid lines) with time (day of the year). Data points with error bars represent the mean ±95% confidence limits (n=6); data points without error bars indicate that the plasma of the six individual fish was pooled prior to analysis. Data are from season 2 fish.

trained groups (Figures 3.7 and 3.9). Statistical comparison of haematocrit between the seasons when the dates of sampling occasions overlapped revealed a statistical difference ($p<0.05$, one way ANOVA), with season 2 fish recording lower mean values. Haematocrit increased steadily in very young fry and fingerlings (Figure 3.9). The datum point indicated by the arrow in Figure 3.9 is the haematocrit value from six alevins (yolk sac external), and indicates that haematocrit is low in recently hatched fish.

Exercise training had a variable effect on blood haematocrit. Continuous training significantly increased haematocrit during all six trials of season 2 ($p<0.01$, one way ANOVA;

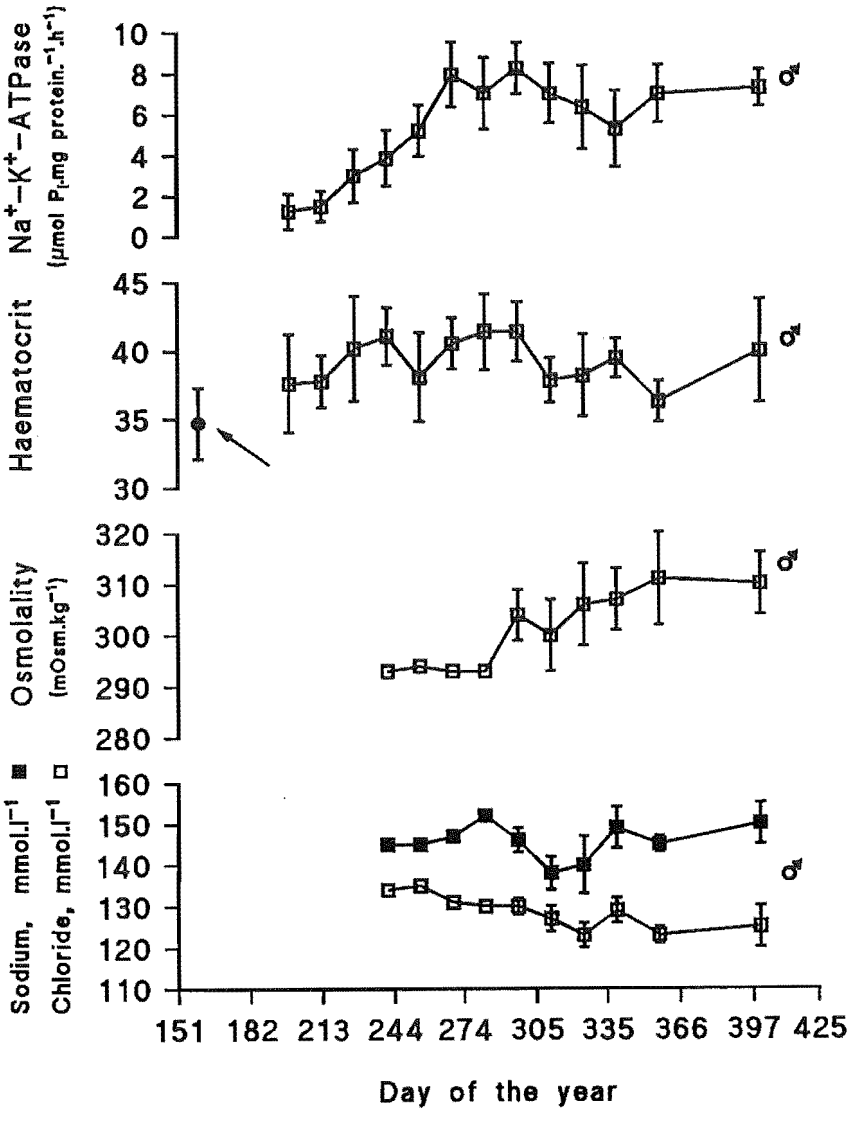


Figure 3.9 Variation in gill Na^+-K^+-ATPase activity, blood haematocrit, plasma osmolality, and plasma concentrations of sodium and chloride in untrained fish from season 3, with time (day of the year). Data points with error bars represent the mean ±95% confidence limits (n=6, except the points marked by the male symbol, ♂, where n=5). Data points without error bars indicate that the plasma of the six individual fish was pooled prior to analysis. The datum point indicated by the arrow is discussed in the text.

Figure 3.8). The periodic training regime (8 hours.day^{-1}) imposed in season 3 did not have the same conclusive effect (Figure 3.10). Trained fish had a significantly higher ($p < 0.05$, unpaired t-test) haematocrit in the first two trials of season 3. Thereafter, untrained and trained groups recorded equivalent results, except for the last two trials, where trained fish had a significantly lower haematocrit than the measured in the untrained fish.

The values for plasma osmolality from fish in season 2 are lower than recorded in season 3 fish (Figure 3.8). However, no overlap of sampling dates occurred, and therefore the low values recorded in season 2 (February and March) may reflect a natural fluctuation in

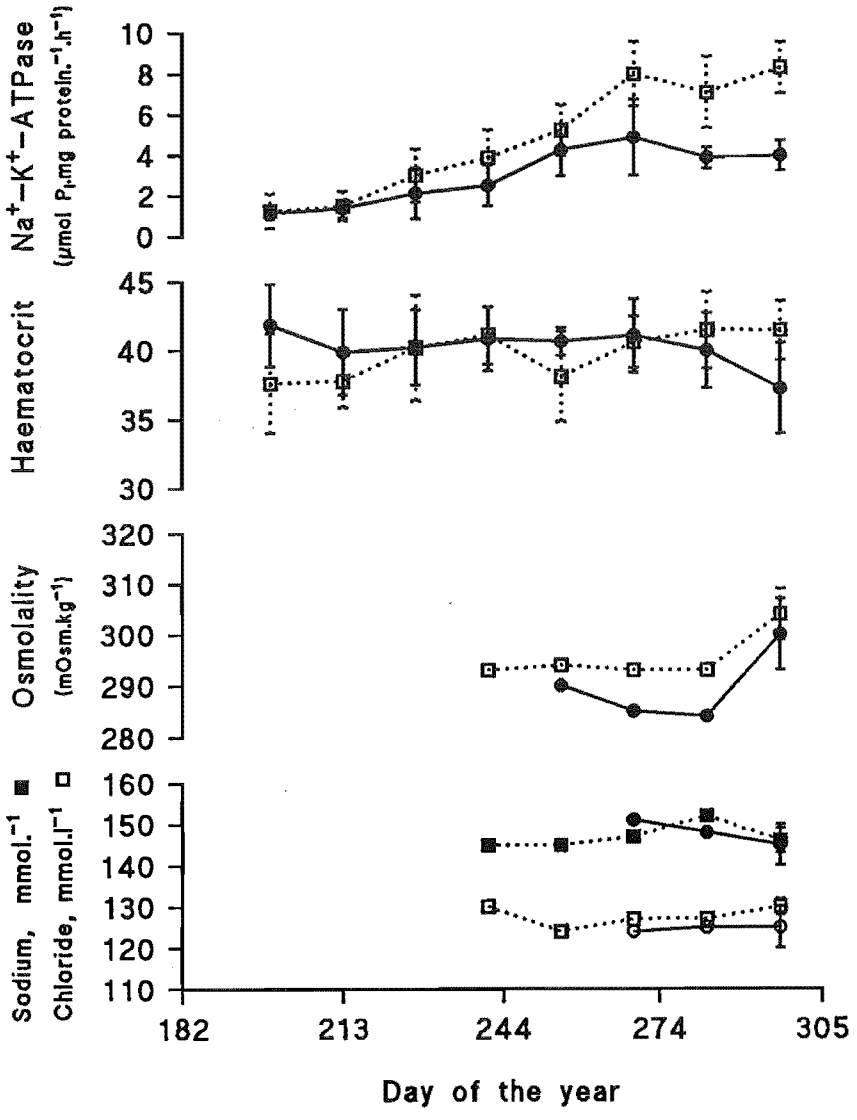


Figure 3.10 Comparison of gill Na^+-K^+-ATPase activity, blood haematocrit, plasma osmolality, and plasma concentrations of sodium and chloride in untrained fish (open symbols, dotted lines) and exercise trained fish (solid symbols, solid lines) with time (day of the year). Data points with error bars represent the mean ±95% confidence limits (n=6); data points without error bars indicate that the plasma of the six individual fish was pooled prior to analysis. Data are from season 3 fish.

plasma osmolality at that time of year. In season 3 (Figure 3.9), plasma osmolality (pooled samples) was low (approximately 293 mOsm.kg^{-1}) in young fry and fingerlings. Osmolality increased thereafter to around 300-305 mOsm.kg^{-1}. Training caused an apparent decrease in plasma osmolality in season 3 (Figure 3.10), although statistical analysis was not possible due to pooled plasma.

The concentration of plasma sodium was generally greater than plasma chloride (Figure 3.7 and 3.9), although both were variable. Both decreased during the time of parr-smolt transformation, but neither remained low for the entire period. As with osmolality, exercise

training apparently reduced the plasma concentration of both electrolytes, although once again, statistical analysis was not possible due to pooled plasma.

DISCUSSION

Three different exercise training regimes were used in this study in an attempt to promote growth in underyearling, hatchery reared, chinook salmon. In total, one long term, and 14 short term, separate exercise training trials were performed and analysed. Growth and growth rate performance were assessed by measurement of final fish size, change of fish condition, and the rates of length and weight increases per day, at the end of each trial. Although the fish used in each season were siblings, derived from the same hatchery population, and were randomly selected to each group at the start of each trial, the initial group variances of untrained and trained fish were checked for homogeneity. No differences of variance were found between the untrained and exercise training groups of any trial, and therefore the changes that occurred at the end of each trial are assumed to have resulted from the experimental treatments (*i.e.* exercise trained *versus* untrained).

Despite the plethora of information to the contrary (Davison, 1989; Farrell *et al.*, 1990), exercise training of underyearling chinook salmon did not enhance the growth rate of the trained fish in any trial. The long term training trial (season 1, one untrained and three trained groups) produced fish of equivalent size after 85 days of training. These fish however, were significantly smaller and had a lower condition factor than siblings reared under normal commercial farm practices (Figure 3.2). Although the experimental fish were fed at the same frequency as the farm fish, the design of the raceway and the anterior position of the feed hopper may not have facilitated even feed distribution with respect to the spatial distribution of the fish (Wankowski and Thorpe, 1979a). The group variances for length and weight increased throughout the study, indicating dominance hierarchies and a spread of individual growth rates. In addition, the experimental fish were subjected to frequent potential stressors; repeated handling and anaesthesia, and periodic reductions in water flow due to blockage by weed. If the experimental fish were 'stressed' by these factors, growth was likely to have been compromised (Pickering, 1981).

Moreover, weight specific (SGR) and linear growth rates (LGR) of 1-2 gram, untrained fish from season 2 and 3 were considerably higher (around 5% body weight.day^{-1}, and 0.7 mm.day^{-1} respectively) than in similarly sized untrained and trained fish of season 1 (1.7% body weight.day^{-1}, and 0.4 mm.day^{-1} respectively). This may be further evidence that the experimental design of season 1 caused all the fish to be stressed, and thereby reduced their growth.

Trained fish were significantly smaller and in 'poorer' condition than untrained fish at the end of every short term trial in season 2 and 3 (Figure 3.3) The specific, and linear growth rates of trained fish were significantly less than in untrained fish groups in every trial. It was

interesting that linear growth rates of young fry were initially low with respect to the fastest rates recorded in the fingerling and smolt stages. The concurrent low rate of linear growth, and high specific growth rate, caused an alteration of fish appearance from the long and 'skinny' fry to the more 'fish like', fuller bodied, fusiform fingerling. Furthermore, faster linear growth rate (and lower specific growth rate) over the period of parr-smolt transformation would lead to more slender and streamlined fish; such terms are frequently used to describe smolts (Vanstone and Markert, 1968; Fessler and Wagner, 1969; Wedemeyer *et al.*, 1980; Folmar and Dickhoff, 1982; Hoar, 1938, 1988). Condition factor alone is unlikely to indicate such relative changes of body morphology. The detailed measurement of many standard morphometric characters would be necessary.

The growth rates (and growth curve, Figure 3.1) of untrained fish recorded here are similar to those in the literature. Kjelson and co-workers (1982) reported the linear growth rate of tagged juvenile chinook salmon of the Sacramento River (ancestral stock) as 0.26-0.40 mm.day^{-1} for fish resident in freshwater, and 0.40-1.23 mm.day^{-1} for fish resident in the Sacramento-San Joaquin estuary. Healey (1980) estimated the linear growth rate of juvenile chinook in the Nanaimo Estuary to be 1.32 mm.day^{-1}. Linear growth rates of 0.28-1.01 mm.day^{-1} were estimated by Carl (1984) for chinook resident in two Lake Michigan tributaries (chinook were successfully introduced and established in Lake Michigan in 1967). Unwin (1986), calculated the growth rate of wild juvenile chinook salmon of the Glenariffe Stream as 0.29 mm.day^{-1}.

In underyearling Atlantic salmon, eventual upper mode, 1+ smolts maintain faster growth over the summer and autumn months than eventual lower mode, 2+ smolts in their first year of life (Thorpe, 1977; Thorpe and Morgan, 1978a, 1980; Thorpe *et al.*, 1980; Higgins and Talbot, 1985; Thorpe, 1989). Growth rate during the parr-smolt transformation is rapid in smolts relative to non-smolting fish (Saunders and Henderson, 1970; Komourdjian *et al.*, 1976b). Such conclusions can not be drawn from the data presented here for chinook salmon with regard to specific growth rate; which declined steadily with time. Linear growth rate on the other hand, was indeed fastest during the period of parr-smolt transformation.

Exercise training of wild chinook produced similar reductions in growth rate as found with their hatchery counterparts (Table 3.1). Specific and linear growth rates were significantly reduced in the trained group. Additionally, the wild, untrained fish did not grow as fast as hatchery untrained fish of the same size. This may be in part due to the stress associated with wild fish experiencing an unfamiliar environment in the laboratory, particularly confinement to a restricted space, intense illumination/lack of cover, and the (probable) increase in fish proximity and density. Equally, the change to inanimate prey may have reduced feeding frequency and efficiency.

Although it is impossible to determine what the growth rate of the wild fish was prior to capture, peak fry emergence in Glenariffe Stream occurs in September (fry may be caught from August to early November; Unwin, 1981). The wild fish used in this study (initial measurements of 72.62 mm and 3.73 g) were caught in late November and using Unwin's

(1986) size ranges for the fry and smolt stages of growth, should be called smolts (*i.e.* two to three month old fish). Equivalent sizes of two and three month old hatchery fish for season 3 were approximately 48.93 mm and 1.05 g, and 71 mm and 3.89 g, respectively. Therefore, although Unwin (1986) gave a value for the linear growth rate of wild fish of 0.29 $mm.day^{-1}$, somewhat lower than the hatchery fish in this study, the wild fish may well have been growing at a similar rate to the hatchery fish prior to capture. Periodic manual feeding meant that feed availability was unpredictable, as would be case with prey organisms in the wild, where chinook are predominantly selective feeders, targeting active and 'vulnerable' prey items (pupae and emerging adults of chironomids and tricopterans), rather than randomly striking at the entire invertebrate drift (Sagar and Glova, 1987, 1988). This could partly explain why they did not 'gorge' themselves at feeding, as was the way of hatchery fish.

Whilst condition factor of both untrained and trained fish increased throughout the periods of investigation, the index was consistently lower in the latter at the end of each trial. Furthermore, in contrast to Atlantic salmon (Farmer *et al.*, 1978; Virtanen, 1987; Soivio *et al.*, 1988), coho salmon (Vanstone and Markert, 1968), and steelhead trout (Fessler and Wagner, 1968), a decrease in condition factor over the period of parr-smolt transformation was not recorded. As the fish used in this study were reared under artificial, hatchery conditions, and in warmer than normal water, it is unlikely that such a decrease would be noted (Johnston and Saunders, 1981; Virtanen *et al.*, 1981; Folmar and Dickhoff, 1982). However, Unwin (1986) documents for wild chinook, that there is a definite increase in fish condition of fingerlings (smolts?) compared to fry, during the outmigration from Glenariffe Stream. Exercise training (8 $hours.day^{-1}$) of sockeye salmon did not produce the marked reduction in growth rates or final size in the trained fish after 26 days of training. Mean length and weight of trained fish were less than for the untrained fish however.

Is this the first study of exercise training and growth to report such a strong negative result? Recourse to the literature reveals that there have been a few papers published that have reported similar findings to this study, *i.e.* no effect, or growth retardation of trained fish compared to untrained fish. These studies used low intensity (1-2 $bl.s^{-1}$), long duration (month or longer) swimming for the exercise training period. Davie and co-authors (1986), working on rainbow trout, recorded differing mean weights for control (untrained) and trained fish in Tables 1 and 4 of their paper (Davie *et al.*, 1986). Comparison of final weights for trained and untrained fish in Table 1 (their paper) gives the impression of a large increase in weight as a result of 200 days training. In Table 4 (of their paper), the untrained fish value is larger than for the trained fish, and there is obviously no significant difference. Interestingly, the authors make no comment in their discussion regarding a significant increase or decrease in fish size as a result of training. They do report however, that there was no difference in fish condition between the groups following training, the opposite to what was found here with chinook salmon.

Similarly, Farrell and co-authors (1991), reported a decrease in mean weight of trained rainbow trout after a 28 day exercise period (Farrell *et al.*, 1991). The control fish grew well,

and were significantly larger and in better condition than the trained group at the end of the experiment. Again, no mention of a *reduction* in growth rate is given in the text. Rather, the result is written positively in terms of growth of the 'control' (untrained) fish. The paper on Arctic charr by Christiansen and associates (1989), states that the growth of trained fish was faster than in still water controls. However, this was never statistically significant between the groups throughout the entire experimental period. Greer-Walker and Emerson (1978) also produce data purporting to enhanced growth with exercise, unsubstantiated by statistical significance. Furthermore, the mean weights of 2 and 3 $bl.s^{-1}$ trained fish (rainbow trout) in their paper were lower than their respective controls, and although the mean weight of the 1 $bl.s^{-1}$ group was larger than the control group, the standard deviations of both, completely overlapped. In one other paper, Shaw and associates (1975b) found that growth rate (over a period of two months) of fresh- and seawater resident Atlantic salmon was unaffected by training at approximately 1 $bl.s^{-1}$.

Both untrained and trained fish were normally found with feed in their guts upon dissection. Untrained fish generally had a fuller gut than the trained fish, and as undigested feed contributed to total body weight, a 'carcass' condition factor was calculated and used to compare trained and untrained fish. This index is the same for the 'normal' condition factor, except that carcass weight (body weight minus viscera weight) was used instead of total body weight. Despite this modification, untrained fish had a significantly higher condition (Figure 3.3).

No attempt to determine feed consumption, or feed conversion efficiency rates of either group were made during this study. Fish were fed to satiation, and this was routinely noted when additional presentation of feed was not taken. The faster growth recorded by the untrained fish may have been due to increased feed consumption. It is not known whether training had any effect on digestion and evacuation rates of feed, although quicker processing would increase the likelihood of any empty gut, post mortem.

Growth of sockeye is much slower than in chinook, for equivalent sized fish. The sockeye and chinook (season 3) were derived from stripping of adults in March and June 1991 respectively. The rearing practices for both species were identical. At the start of the sockeye training trial the sockeye were almost seven months old (post hatching), and weighed 6.68 ± 0.45 (mean $\pm$ SE, $n=6$) grams. The weight of untrained chinook at the same time was 6.69 ± 0.23 grams. The chinook were only five months old (post hatching) at the time. Growth rates of exercise trained sockeye salmon were not significantly less than in untrained sockeye (Table 3.2). However, the mean rate for both linear and specific growth was lower in the trained group. From weight and length data given by Brett and fellow workers (Brett *et al.*, 1969, Table 1 of their paper), linear and specific growth rates of laboratory reared (15 °C), underyearling sockeye were calculated as 0.64 $mm.day^{-1}$, and 1.89 %body $weight.day^{-1}$ respectively, which are considerably higher than in this study.

Morphometric changes of the standard length and length of the caudal peduncle were recorded in this study (Figure 3.4). Standard length was approximately 88% of fork length in 39.86 - 60.67 mm fish, and 90% in larger fish thereafter. The caudal peduncle accounted for

approximately 13% of fork length in fry. It increased, reaching a maximum of around 14.5% in 'smolts', and then returned to 13% of fork length. The increase in both indices occurred at the onset of parr-smolt transformation, although neither were correlated with seawater survival (*see* CHAPTER 6). Increase in caudal peduncle length during the period of parr-smolt transformation has also been reported in Atlantic salmon (Nikolskii *et al.*, 1947), coho and chinook salmon (Winans, 1984; Winans and Nishioka, 1984), and is used to describe the 'smolt' stage as being more streamlined and slender than the 'parr' stage. Further study of the changes in caudal peduncle length and standard length, and the measurement of other morphometric characters, possibly using a sophisticated digitizing technique (Winans, 1984), is therefore warranted.

Scaling of body organ weights with animal growth has been widely recorded, and the whole phenomenon has been thoroughly discussed by Schmidt-Neilsen (1984). Both heart and viscera weight scaled with body weight over the range (Figure 3.5). Training had no effect on the heart, but significantly reduced viscera weight, although this was probably a reflection of there being less feed in the gut of trained fish at death. Previous studies have found exercise training increases heart growth and weight (Hochachka, 1961; Greer-Walker and Emerson, 1978; Davie *et al.*, 1986; Houlihan and Laurent, 1987). Davison (1989) advised that training periods of 3 weeks duration or longer are necessary to produce significantly measurable changes of physiology or morphology. Additionally, the training regimen must be strenuous enough to reach a 'threshold' limit that will cause change. The short term, low intensity training regime used in this study therefore, was probably not sufficient to bring around noticeable changes of heart size.

Water contents of the viscera, heart, and white muscle were not constant with time (Figure 3.6). Water content of the white muscle decreased to a constant value of around 78-79%. Fry (less than one gram body weight) recorded higher water content in white muscle than in all other older fish. The water content dropped sharply to around 77% during the parr-smolt transformation, as has been recorded elsewhere (Virtanen, 1987). Heart muscle averaged 80% water content over the size range, significantly higher than the other two tissues. Whether this high water content was due to moisture from blood in the heart and coronary vessels is not known. Blood was blotted from the heart as much as possible on absorbent tissue prior to weighing and drying. It would have been interesting to determine the water content of red muscle (another well perfused tissue) in this regard. Visceral water content exhibited the greatest decrease with fish growth. Fat was probably responsible for much of the decrease in water content of the viscera. Adipose deposits lined the organs of the gut, becoming increasingly larger and obvious as the fish grew. Upon drying, the dehydrated guts of the larger, post-smolt fish were inclined to be 'greasy', with some having actual pools of liquefied lipid.

The pattern exhibited by gill Na^+-K^+-ATPase activity (Figures 3.7 and 3.9) was similar to those found in other studies, and showed the 'classic' transient rise in activity during the period of parr-smolt transformation (Wedemeyer *et al.*, 1980; Folmar and Dickhoff, 1982;

Hoar, 1988; Franklin, 1989). High rates of gill Na^+-K^+-ATPase activity are one of the factors known to be essential for survival and growth in the marine environment. Exercise training markedly reduced this enzyme activity (Figures 3.8 and 3.10), particularly during the period of parr-smolt transformation. Untrained and trained fish were not exposed to a seawater challenge test to determine their hypo-osmoregulatory ability at the end of a training trial. The results of such a test would have been quite illuminating, and is worth further study, particularly on a larger scale, or commercial basis.

Mean haematocrit of overlapping sampling occasions was lower in season 2 than in season 3. The reason for this is unknown, although different anaesthetics were used. Phenoxyethanol was used in the early part of season 2, benzocaine was used for the last three trials of season 2 and for all of season 3. The change of anaesthetic was because phenoxyethanol was found to clot blood in the gills of seawater transferred fish (*see* CHAPTER 2). Soivio and associates (1977) compared the effects of tricaine methane sulphonate (MS222) and benzocaine on various blood constituents of rainbow trout. They reported that fish anaesthetised with benzocaine recorded the greatest haematocrit values.

Blood haematocrit may be used as a non specific indicator of stress in fish (Schreck, 1982b). It may be argued that the training regimes imposed on the fish were stressful, though this is a mute point, for perhaps the 'still water' situation would constitute an unnatural, and therefore stressful environment for the fish. Young salmon avoid continuous swimming in the wild (Kerr, 1971; McNeish and Hatch, 1978). In any event, training of fish in season 2 (Figure 3.8) caused a noticeable increase in haematocrit over the untrained fish. This was not apparent during season 3 (Figure 3.10), when a variable response was noted. In the first two trials of season 3 haematocrit was significantly greater in the trained fish. The fish in these trials were in the later stages of 'zip-up' fry. Such fish have been reported as having poor swimming ability (Thomas *et al.*, 1969). Haematocrit was equivalent in the following four trials, but training values of haematocrit were higher in the last two trials (Figure 3.10). Seawater challenge tests conducted on sibling fish at the same time, found that these fish were able to successfully adapt to a marine existence (*see* CHAPTER 5). Tentatively, the fish in these last trials could be termed 'smolts'.

The transformation from parr to smolt is thought to be stressful for the young salmon whilst it remains in freshwater (Hoar, 1988), and coupled with the added stress of an enforced exercise training regime, may have caused the increase in haematocrit. The first three values for haematocrit in season 2 (Figure 3.8) were obtained from 'smolts', and were indeed greater in the trained group. The last three values were from large fish (30 grams plus), and these fish may have been slightly overcrowded within the swimming compartment.

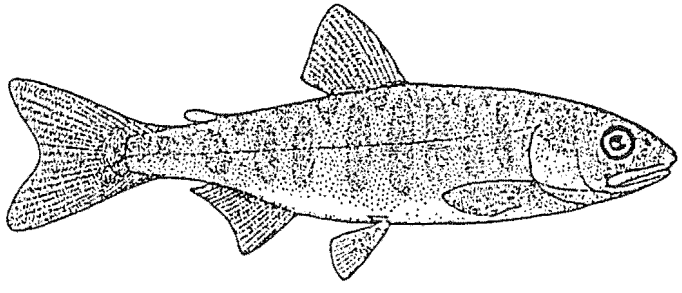
Osmolality in the large fish of season 2 was lower than in season 3, and may be a circannual phenomenon (variation in plasma osmolality with time was recorded by Franklin (1989) in previous studies of chinook obtained from the same stock, *i.e.* the Glenariffe hatchery population). Plasma concentrations of sodium and chloride ions were also quite variable with time. The concentration of sodium was usually greater (approximately 140-150 $mmol.l^{-1}$) than

chloride (120-130 mmol.l^{-1}) throughout the periods of examination. The pooling of blood plasma from the smaller fish prevented statistical analysis of the effect of training on these blood constituents. It would appear that training may have reduced plasma osmolality and both plasma ion concentrations. Wood and Randall (1973a,b,c) have also reported that plasma sodium was reduced in exercised rainbow trout. Previous authors (Virtanen, 1987; Franklin, 1989) have reported decreases in plasma sodium and chloride ion concentration, and percentage water content of white muscle during the period of the parr-smolt transformation. Such perturbations are said to be as a result of osmoregulatory unbalance in the smolt (Thorpe, 1984; Virtanen, 1987). The data are not sufficiently complete in this study to categorically say that this was noted, although similar trends were noted.

Overall, the data for growth rates and gill Na^+-K^+-ATPase activity resulting from exercise training are quite pertinent to the commercial farming industry. The small, laboratory based experiments undertaken in this study caused decreases in growth rates, and lowered the 'normal' rise in gill enzyme activity: changes that are in direct conflict to the goals of salmon farming/ocean ranching businesses. A (necessarily) long term, farm based study of exercise training would be worthy of investigation, notice given by the fact that farm based, exercise training studies (Kuipers, 1982; Leon, 1986; Totland *et al.*, 1987) have provided positive results in the past.

CHAPTER FOUR

CRITICAL SWIMMING SPEED (U_{crit}) OF FRY AND FINGERLING CHINOOK (*Oncorhynchus tshawytscha* Walbaum), AND SOCKEYE (*O. nerka* Walbaum) SALMON



CHAPTER FOUR

CRITICAL SWIMMING SPEEDS (U_{crit}) OF FRY AND FINGERLING CHINOOK (*Oncorhynchus tshawytscha* Walbaum), AND SOCKEYE (*O. nerka* Walbaum) SALMON

INTRODUCTION

Investigations of fish swimming behaviour have been many and varied, dating as far back as Aristotle or before (Lindsey, 1978; Blake, 1983). That fish swim has never been in question; however the means of propulsion, and power output, has provoked much research. Despite the diverse nature of the entire group, swimming modes have been divided primarily into two categories with respect to body movement; (i) undulatory swimming, and (ii) 'non-body' swimming (Blake, 1983).

Undulatory swimming included modes whereby thrust is attained from sinusoidal movements of the body and caudal fin. The waveforms propagate in an anterior-posterior direction (opposite to the direction of animal movement), and increased in amplitude toward the caudal fin. Undulatory swimming was first divided into three modes; anguilliform, carangiform and ostraciiform, by Breder (1926). The carangiform mode has since been sub-divided to include subcarangiform, and thunniform (Blake, 1983). Non-body swimming encompasses all swimming modes in which the body is held rigid, and propulsion achieved by either oscillations of the pectoral fins, or by undulations of the caudal, dorsal, anal, and/or paired fins. Breder (1926) described non-body swimming with seven modes, all of which have currency today (Lindsey, 1978; Blake, 1983).

Mode of locomotion in fishes is clearly associated with form, function, and natural history. Salmonids are described as elongate, fusiform fish, that can maintain steady state swimming with a subcarangiform mode of locomotion. Manoeuvring and short 'bursts' of movement, particularly at low velocities, are directed through movements of the paired (particularly the pectoral) and median fins.

Research has investigated swimming ability of fishes in human terms - through performance tests of maximum (burst) speed, and stamina. Whilst certain taxonomic Families fare well in such analyses, others with a sedentary life history would fare badly. Performance studies of swimming ability are therefore most suited to 'natural swimmers'; the pelagic and migratory fish. For a review of the literature, see Beamish (1978) and Davison (1989).

There are numerous theoretical studies of fish swimming behaviour (Beamish, 1978). Much of the early work was mathematical in nature, attempting to explain the mechanics and kinematics of locomotion, with algebraic equations and mathematical models. The advent of cinematography, has led to much research on animal locomotion kinetics (Marey, 1894). Recent analysis of the theoretical equations with data from free swimming fish has shown good correlation (Webb and Weihs, 1983).

In recent years, the swimming performance of many fish species has been tested using a variety of methods. Blažka and associates (1960), and Brett (1964) described laboratory equipment ('swim-tunnels') that was designed to force fish, enclosed in a compartment, to swim at known velocities. Furthermore, the velocity of the water flow could be increased and decreased to allow measurement of various parameters at different, known swimming speeds/work loads.

Performance of swimming has been measured and documented using a number of parameters, such as maximal swimming speed (MacLeod, 1967; Wardle *et al.*, 1989), burst speed (Weaver, 1963; Weihs, 1974; Videler and Weihs, 1982; Pucket and Dill, 1984; Taylor and McPhail, 1985a,c; Wardle and He, 1988), migratory cruising speed (Johnson, 1960; Ellis, 1966; Smith *et al.*, 1981; Laughton, 1989; Webb, 1989; Webb and Hawkins, 1989), time to fatigue at prescribed water velocities (Bainbridge, 1960, 1962; Brett, 1964; He and Wardle, 1988), and critical swimming speed (Brett, 1964).

In this chapter, the definition of swimming activities follows the terminology of Hoar and Randall (1978; preface to volume seven). *Sustained swimming* refers to a spectrum of swimming activities and velocities that can be maintained for an indefinite period (in practical terms, longer than 200 minutes). Sustained swimming as defined, is necessarily aerobic, and does not cause fish fatigue. Sustained swimming is most often used to describe the natural swimming of wild fish, and encompasses foraging, station holding, schooling, and general steady cruising, during migration. *Burst swimming* refers to short term (less than 15 seconds), anaerobic swimming. This category may be further subdivided into an acceleration period (often from a 'standing' start), and a 'sprint', when swimming velocity is high, but steady. Burst swimming may be observed at times of prey capture, or predator avoidance, and when fish are startled.

Between the extremes of sustained and burst swimming is the category *prolonged swimming*. As defined, prolonged swimming covers a spectrum of swimming speeds and activities that are longer than 15 seconds, and less than 200 minutes. Energy for such swimming is derived from both aerobic and anaerobic sources, and as such, will end in fatigue if maintained over long periods. Fatigue associated with prolonged swimming is the concept behind high-seas trawling (Wardle, 1977). *Fatigue* is observed when a fish 'collapses', fails to maintain swimming at a given water velocity, and is swept back by the water current.

Critical swimming speed (U_{crit}) is a specific 'operational' term used to describe measurements of prolonged swimming in the laboratory. In this way, swimming performance may be compared between species and between individuals of the same species, with different physiological states. The measurement of U_{crit} is restricted by certain 'rules' of methodology which was first defined by Brett (1964). Fish are forced to swim at a known water velocity for a prescribed period of time (normally within a swim-tunnel); the water velocity is increased by known increments at predetermined time intervals until the fish fatigue. Critical swimming speed is then computed as the maximum swimming speed achieved prior to fatigue, and is calculated by interpolation for those fish that fatigue at some time during each water velocity/time interval period.

Brett (1964), working on sockeye salmon (*Oncorhynchus nerka*), described the methodology for determining critical swimming speed as a definitive laboratory based measurement of swimming performance. Since his pioneering work, there have been many studies measuring critical swimming speeds of a great many fishes, however, the exact methodology followed by each worker has generally varied from that originally described by Brett (1964), reducing the validity of comparison between studies (references given here are for salmonids only: Thomas *et al.*, 1964; Brett, 1967; Brett and Glass, 1973; Jones, 1971; Webb, 1971b; Griffiths and Alderdice, 1972; Jones *et al.*, 1974; Farlinger and Beamish, 1977; Glova and McInerney, 1977; Smith, 1982; Flagg and Smith, 1982; Besner and Smith, 1983; Flagg *et al.*, 1983; Taylor and McPhail, 1985a,c; Farrell *et al.*, 1990, 1991; Butler *et al.*, 1992; Gallaugher *et al.*, 1992; Kolok, 1992).

Swimming performance has been assessed using other criteria. Smith and co-workers (Smith, 1982; Flagg and Smith, 1982; Besner and Smith, 1983; Flagg *et al.*, 1983) described swimming performance in terms of 'stride length' and tail beat frequency (TBF) in fingerling and smolt stages of coho salmon (*Oncorhynchus kisutch*). It was shown that the swimming stamina (U_{crit}) of coho decreases in fish directly transferred to seawater, and remains reduced for up to three weeks post transfer (Flagg *et al.*, 1983). In conjunction with the loss of stamina, TBF of smolts was higher than that of parr at the same water velocity (equivalent to four body lengths per second), and smolts were therefore far less efficient swimmers (Flagg and Smith, 1982). Smith (1982) has hypothesised that the decrease in swimming stamina was a necessary component of the downstream migration of smolts, which has been shown to be largely a passive movement (Arnold, 1974; Raymond, 1968, 1979; McCleave, 1978; Thorpe *et al.*, 1981). Atlantic salmon smolts have a much reduced swimming ability compared to parr (Thorpe and Morgan, 1978b).

Expansion of the leisure industry, professionalism in sport, and a greater awareness of 'sports fitness' for improved health, has increased research output on the effects of exercise training on cardiovascular, muscular, and athletic performance in humans (Clausen, 1977; Blomqvist and Saltin, 1983; Hoppeler, 1986; Wilmore and Costil, 1988; McArdle *et al.*, 1991). Human and mammalian studies of exercise training and its effect on performance are subject to a strict code of 'rules' with respect to the bout intensity, duration, and type of exercise undertaken, allowing for direct comparison (Clausen, 1977; Blomqvist and Saltin, 1983). There have been several conferences, symposia, and reviews dealing specifically with exercise training, physical conditioning, and sports medicine, the most recent by Hill (1993).

Exercise training research is essentially of two types: cross-sectional and longitudinal. Cross-sectional studies compare control subjects with trained athletes, and measure the genetic components that contribute toward athletic performance. Cross-sectional studies have relevance in fish exercise physiology, allowing for performance comparison between wild and hatchery stocks, or between differing strains of hatchery reared fish. Longitudinal studies compare performance of the same subjects before and after a training regime, and thus examine the effects of the exercise regime itself. Longitudinal studies have been more common in fish

exercise research (Davison, 1989), although comparison between studies is often limited due to varying methodology, equipment, and objectives.

This study had several objectives, and involved both cross-sectional and longitudinal investigations. The primary objective was to determine the critical swimming speeds (U_{crit}) of underyearling chinook (*Oncorhynchus tshawytscha*) during growth from alevin to post-smolt. The effect of a ten day, low intensity exercise training regime on subsequent U_{crit} performance was also investigated. Two differing training regimes (one more exacting than the other) were used. Critical swimming speeds were measured for sibling groups of trained and untrained fish during the first nine months of life. U_{crit} tests were also carried out on seawater resident fish to determine whether swimming ability was altered as a result of growth in seawater. The seawater fish were allowed at least a week to adapt to a marine environment before their critical swimming speeds were determined. It was also determined whether the swimming performances of individual fish were repeatable. Three measurements of U_{crit} for each individual were made over a twelve hour period. The percentage change in swimming performance was calculated for the second and third trials of each fish.

The U_{crit} performance of underyearling chinook and sockeye salmon (*O. nerka*) were compared. A group of 'wild' (*i.e.* naturally spawned) underyearling chinook was also subjected to U_{crit} tests, and their swimming performance was compared to that of the hatchery chinook. Various physiological parameters were measured immediately after completion of the U_{crit} test. They were compared between the groups above and to respective values for resting untrained, 'control' fish.

MATERIALS AND METHODS

Fish stocks and general husbandry

Hatchery reared chinook (*Oncorhynchus tshawytscha*) and sockeye (*O. nerka*) salmon underyearlings were obtained from the Ministry of Agriculture and Fisheries' (MAF) Glenariffe Hatchery. Fish were transported to the Zoology Department (hereafter the Department), measured for fork length and wet weight, and randomly assigned to one of three groups - exercise trained, untrained U_{crit}, and untrained resting fish - six fish per group. Hatchery chinook, from two consecutive brood stock years, were collected periodically during July - March. Wild chinook underyearlings were collected on one occasion (23 November 1991) and were allowed to recover for 60-70 hours prior to being subjected to the U_{crit} test. Sockeye were collected on one occasion (25 October 1991) and reared at the Department for up to four months prior to U_{crit} measurement.

Exercise training

The exercise trained fish were placed into a 175 litre, oval 'racetrack' flume (*see* CHAPTER 2, Figure 2.4 and surrounding text). In season 2, the training group was forced to swim against

a continuous current of 1-1.5 body lengths per second ($bl.s^{-1}$) for 10 days. In season 3, the training set up was much the same as for season 2, except that the training regime was reduced to 8 $hours.day^{-1}$. Both groups of untrained fish were placed into glass tanks. The flume and the glass tanks had constant flows of artesian water; good circulation and aeration was ensured by gently bubbling compressed air through air stones. All fish were fed to satiation at least five times daily, and were subjected to individual U_{crit} tests at the end of the training period. Fish were not starved prior to the U_{crit} tests, and were fed on the day that swimming performance (U_{crit}) was measured.

Routine measurement of U_{crit}

The fish 'tunnel' respirometers used were based on the design of Blažka and co-workers (1960; *see* CHAPTER 2, Figure 2.5 and surrounding text). All the U_{crit} tests were performed with only a single fish in the swim-tunnel at a time. Tests were performed in both freshwater and seawater. Seawater tests were performed on fish that had been resident in seawater for at least a week. Fish were taken from the tanks or flume, lightly anaesthetised, and measured. During recovery, they were placed into the 'tunnel', and this was secured to the impeller casing. The respirometer was filled with water and the lid tightly sealed. Each fish was allowed to recover for at least 10 minutes prior to the start of the test. During this time, the fish regained balance, and explored the confines of the tunnel.

To start the test, a water velocity equivalent to one $bl.s^{-1}$ was set, and the fish were required to swim against this current for 10 minutes. Step wise increments of one $bl.s^{-1}$ were imposed on the fish every five minutes thereafter, until the fish fatigued. This methodology differs somewhat from the 10 cm increments and one hour duration time periods suggested by Brett (1967) and Beamish (1978), but was chosen due to the size range of the fish to be studied and time constraints if all the fish in each trial were to be tested. Fish were observed throughout each trial, to ensure continuous swimming, and whether swimming was effected by steady or unsteady movements. Ventilatory rate (gill opercula movements) was determined before and immediately after the test, and was counted in two separate, 10 second periods during the last 30 seconds of each velocity increment.

Exhaustion was observed when the fish failed to maintain its position in the current, being forced backwards on to the screen. If increased electrical stimulation failed to force the fish to continue swimming, the test and the water flow was stopped. Exhausted fish would lie on the screen, or on the 'floor' of the tunnel for up to 30 seconds, ventilating deeply. After this time, they would regain their balance, and rest on the tunnel floor. In some instances, fish 'faked' fatigue, and showed an unwillingness to swim early in the U_{crit} test. Such fish did not behave in the same way as truly exhausted fish. Although repeated electrical stimulation failed to remove them from the grid, as soon as the water current was stopped, the 'fraudster fish' would immediately regain a stationary position on the tunnel floor, or gently swim around in the tunnel. Upon noticing such behaviour, the flow was immediately restarted. In some cases, the fish would return to the downstream grid within 5-10 seconds, and if this happened the trial was

terminated. If, however, the fish took up steady swimming again, the trial was continued. At each subsequent failure, these fish were similarly checked, until exhaustion was evident.

Most fish did not tire exactly at the beginning or end of a prescribed swimming period. For those fish that fatigued during the five minute intervals, the critical swimming speed was calculated by interpolation using Brett's (1964) formula $U_{crit} = v_p+(t_e/t_i \times v_i$ (where v_p is the penultimate velocity, *i.e.* the fastest velocity that was maintained for the whole of the prescribed swimming period; t_e is the time elapsed prior to fatigue at the ultimate velocity; t_i is the duration of the prescribed swimming period; and v_i is the velocity increment. With this equation, the values for v_p and v_i are interchangeable with regard to the real swimming speed ($cm.s^{-1}$) and the relative swimming speed ($bl.s^{-1}$) of the fish.

Four fish were subjected to 'multiple' U_{crit} tests to determine whether there was a 'conditioning' effect on critical swimming speed. Each fish was tested individually and on three occasions. At the end of a U_{crit} test, the fish was netted from the respirometer and placed into a recovery tank for a period of four hours - previously shown to be a suitable length of time to permit full recovery to a 'resting' state (Professor AP Farrell, personal communication, 1991). After all four fish had been tested once, the first of the group was returned to the respirometer box and subjected to the second U_{crit} test. The fish were treated in this way until three separate measurements of U_{crit} had been determined for each fish.

Sampling

At the end of a U_{crit} test, fish were quickly removed from the tunnel respirometer and killed by overanaesthesia, and remeasured. The tail was severed at the caudal peduncle, and blood from the caudal vasculature was collected into ammonium heparinised micro capillaries. The capillaries were centrifuged at 5000 *g* for six minutes, and blood haematocrit measured. The plasma was transferred to plastic vials and snap frozen in liquid nitrogen. The gills were removed, rinsed in homogenising buffer, placed into plastic vials, and snap frozen in liquid nitrogen. All the samples were stored at −80 °C, until analysis.

Physiological analysis

Blood plasma was analysed for sodium and chloride ion concentration ($mmol.l^{-1}$) and osmolality ($mOsm.kg^{-1}$). Sodium concentration was analysed in a Varian Techtron 1200 Absorption Spectrophotometer. Chloride concentration was determined using a Radiometer CMT 10 chloride titrator. A Wescor Inc. 5100C vapour pressure osmometer was used to determine plasma osmolality. Plasma was pooled, as necessary, prior to analysis, to facilitate that all three determinations were measured for each experimental group. Activity of the enzyme Na^+-K^+-ATPase was measured in the gill epithelia, using previously described methods (Johnson *et al.*, 1977; Langdon *et al.*, 1984; Franklin, 1989).

Statistical analysis

Results are presented as individual measurements, or as the group mean ±standard deviation,

or ±95% confidence limits. Linear regression was performed on some of the relative ($bl.s^{-1}$) and real ($cm.s^{-1}$) speed data. Comparisons between the slopes of the untrained hatchery chinook (reference fish in Figures 4.1, 4.3 and 4.4) and those produced by exercise trained, and seawater transferred chinook, were made using a *t*-test (Sokal and Rholf, 1981). The *t*-test compared the slope (*b*: *i.e.* the gradient, or regression coefficient) and its standard error of the reference fish with the slope and standard error of the trained and/or seawater resident fish. Comparisons of this nature were carried out on both the relative ($bl.s^{-1}$) and real ($cm.s^{-1}$) speed data. Statistical comparison of the untrained hatchery chinook with the wild chinook and sockeye salmon were assessed using two and one way analyses of variance (ANOVA) respectively. Comparison of means was done using Student's paired and unpaired *t*-tests. To compare the swimming speeds of untrained hatchery chinook with the sockeye and wild chinook, groups ($n=9$ for sockeye, and $n=6$ for wild chinook) of similar sized (fork length), untrained hatchery chinook were picked at random from the entire reference group data set. Statistical significance was recorded when $p \leq 0.05$.

RESULTS

Fish behaviour in the swim-tunnel

Fish used in this study quickly recovered from the light anaesthesia, and rested quietly on their pectoral and caudal fins on the floor of the tunnel, or quietly swam around and investigated their new environment. When the water flow was started most of the fish immediately orientated themselves into the current and swam steadily. Some 'refused' to swim and were swept back to the electric grid, and then either initiated swimming or lay still. The voltage was rapidly increased (to the maximum 14 volt, DC if necessary) for a brief time to encourage the fish to swim. If this failed, the water flow was stopped, and the fish was allowed to swim off the grid in its own time. The water flow was then restarted. Normally the fish would assume swimming thereafter. No fish totally refused to swim in a U_{crit} test. At the low swimming speeds of 1-2 body lengths per second ($bl.s^{-1}$), the fish would often swim with their tails brushing the electric grid. They would then position themselves upstream a little further to prevent touching the grid. Most fish assumed this rear/low position in the tunnel for most of the U_{crit} test, only straying at the fastest water velocities when the swimming action became more erratic.

Steady swimming was observed throughout the U_{crit} tests, except in fatiguing fish. Normal, steady swimming was characterised by a 'smooth' swimming action, and good position holding without much yawing or pitching. At intermediate swimming speeds, the fish angled their bodies, head down, into the current. The onset of fatigue was observed when the fish assumed a more erratic mode of swimming. Tiring fish were observed to affect a dart-and-drift (also termed burst-and-coast swimming; Videler and Weihs, 1982) type of swimming as they approached exhaustion. Rather than hold a steady position, the fish would increasingly 'roam' within the tunnel, 'darting' to an upstream position and then 'drifting' back downstream. The

dart-and-drift swimming would often return to a more steady swimming mode. This overall swimming pattern would oscillate between the steady and unsteady modes described above, becoming more exaggerated and frantic, and the test would end soon after with the exhausted fish being swept backwards on to the electric grid. 'Head-butting' and attempted biting of the upstream screen was often observed, although such behaviour was generally short lived.

An 'unwillingness' to swim was frequently observed (often preceded by a short period of dart-and-drift type swimming) at quite slow water velocities (1-3 $bl.s^{-1}$). Such fish would be 'unco-operative', resist swimming, and rest on the electric grid, despite efforts to remove them (increased voltage). A change of water speed (at the next speed increment), would normally cause a resumption of steady swimming.

Ventilation rate increased during the U_{crit} tests. The rate was higher in fish recovering in the swim-tunnel before the test than it had been prior to capture from the tank or flume, and therefore the fish could not be considered to be in a resting state prior to the test. In many U_{crit} tests, ventilatory rate did not increase (above the pre-test rate) during the conditioning speed of one $bl.s^{-1}$. The rate increased thereafter, reaching a plateau of around 180-210 ventilatory cycles per second. In approximately 40% of the tests, 'ram ventilation' (Muir and Kendall, 1968) of the gills was adopted by the fish at the faster swimming speeds. The occurrence of ram ventilation was recognised as a considerable reduction or complete cessation of ventilatory rate and buccal pumping.

U_{crit} performance

The repeatability of the U_{crit} test was assessed in four fish that were subjected to multiple (three) determinations of critical swimming performance. The results are shown in Table 4.1. There was no difference between either parameter of U_{crit} performance ($bl.s^{-1}$, or $cm.s^{-1}$; $p>0.05$, one way ANOVA) in any of the trials.

Table 4.1 Repeated measurements of U_{crit} for a group ($n=4$) of untrained, hatchery chinook. The results were obtained over two days. Two fish were tested per day, and were given a four hour recovery period between each U_{crit} determination. The average fork length of the group was 35.91 ± 2.27 mm. Data are given as the mean $\pm 95\%$ confidence limits. The numbers in parentheses represent the percentage increase of performance in that trial relative to the first trial. The were no significant differences ($p>0.05$) in swimming performance between the trials (one way ANOVA).

	CRITICAL SWIMMING SPEED (U_{crit})	
Trial Number	body lengths per second ($bl.s^{-1}$)	centimetres per second ($cm.s^{-1}$)
1	8.0 ± 0.5	28.4 ± 2.9
2	8.5 ± 0.8 (6.3%)	29.7 ± 2.3 (4.6%)
3	8.4 ± 1.2 (5.0%)	29.5 ± 3.7 (3.9%)

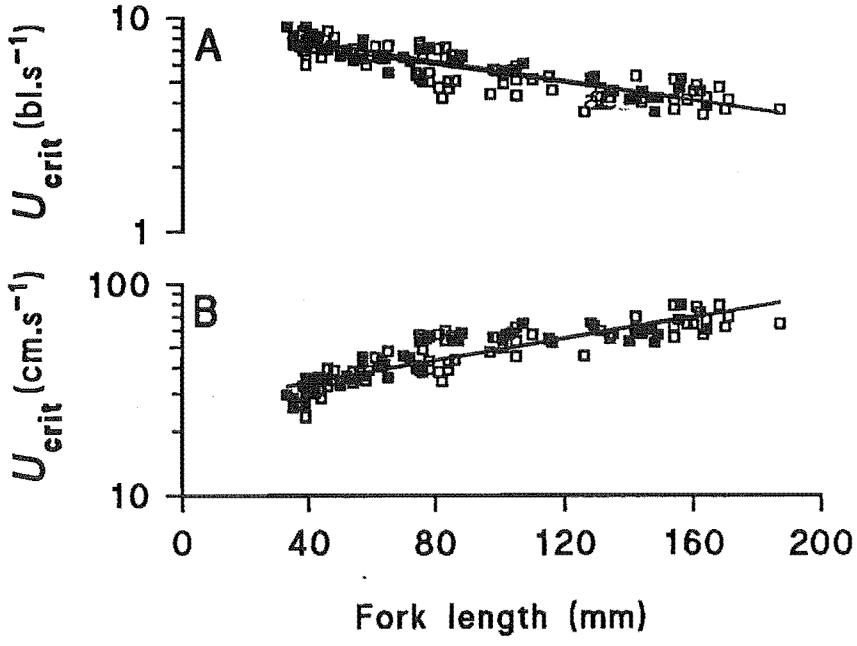


Figure 4.1 Individual measurements of U_{crit}, expressed in terms of body lengths per second (bl.s^{-1}, Figure 4.1A) and real speed (cm.s^{-1}, Figure 4.1B), against fork length (mm) for underyearling chinook salmon. Untrained fish are represented by open symbols, and trained fish by solid symbols. The data for both groups have been pooled from season 2 and 3. The regression lines are given in the text.

Swimming performance of each fish was described in terms of relative speed (bl.s^{-1}) and real speed (cm.s^{-1}), as shown in Figure 4.1. Results from both seasons have been pooled for the untrained and trained fish. Both measurements of swimming speed showed increasing variation and curvilinear relationships with fork length. Therefore, both indicators of swimming performance have been plotted with logarithmic scales along the ordinate. Linear regressions were calculated and compared between the trained and untrained fish.

U_{crit}, expressed as relative speed, declined exponentially with fish size, from a maximum of 9.0 bl.s^{-1} in young 'zip-up' fry (30-35 mm) to around 4.0 bl.s^{-1} in large post-smolt fish (150-180 mm, Figure 4.1A). The data for both the untrained and trained fish have been pooled from season 2 and 3. The regression lines calculated on the relative speed data gave the curves, $\log Y = -2.11 \times 10^{-3}(X) + 0.947$ ($r^2 = 0.734$, $n = 89$), and $\log Y = -2.28 \times 10^{-3}(X) + 0.976$ ($r^2 = 0.823$, $n = 37$) for the untrained and trained fish respectively. Statistical comparison of the slopes of the lines indicated that there was no significant difference between the groups ($p > 0.05$, t-test).

Real speed values of U_{crit} performance increased with fish size. The relationship between critical swimming speed (cm.s^{-1}) and fork length appears to be asymptotic. Real speed U_{crit} for 'zip-up' fry was around 27-30 cm.s^{-1}, and increased, with size, to around 75-80 cm.s^{-1} in post-smolt fish. Real speed swimming performance improved markedly in the fish with fork lengths between 40 and 50 mm, as shown in Figure 4.1B. The regression lines calculated on

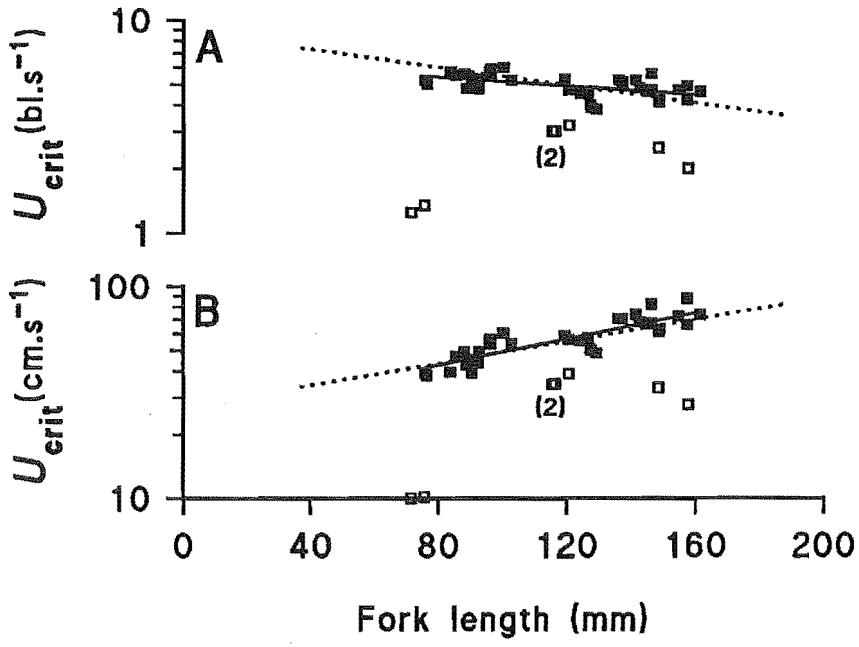


Figure 4.2 Individual measurements of U_{crit} for seawater resident chinook salmon expressed as relative (bl.s^{-1}, Figure 4.2A) and real speed (cm.s^{-1}, Figure 4.2B) against fork length (mm). Solid symbols indicate fish that had grown well in seawater prior to the measurement of U_{crit}, and open symbols represent fish that exhibited poor growth in seawater. The open symbol points have not been included in the regression analysis for this reason. The figure in parentheses indicates that there are two fish at this fork length. The regression lines are given in the text.

real speed data gave the curves $\log Y = 2.56 \times 10^{-3}(X) + 1.43$ ($r^2 = 0.737$, $n = 89$), and $\log Y = 2.64 \times 10^{-3}(X) + 1.42$ ($r^2 = 0.792$, $n = 37$), respectively. Statistical comparison of the slopes of the lines indicated no difference between data sets ($p > 0.05$, t-test). From the data therefore, a decrease in swimming performance was not apparent over the period of parr-smolt transformation (equivalent to fork lengths between 70-110 mm) in either the trained or untrained fish from either season.

U_{crit} tests involving seawater resident chinook (*see* CHAPTER 5 for a detailed description of the protocol involved with seawater transfer) yielded contrasting results with respect to whether or not the fish were 'adapted' to the marine environment (Figure 4.2). Seawater adapted chinook swam as well as their freshwater siblings during the U_{crit} tests, whereas non-adapted fish swam ineffectively, if at all.

The regression lines calculated for the seawater data are given by the equations $\log Y = -7.22 \times 10^{-4}(X) + 0.774$ ($r^2 = 0.141$, $n = 35$, Figure 4.2A) and $\log Y = 3.01 \times 10^{-3}(X) + 1.39$ ($r^2 = 0.743$, $n = 35$, Figure 4.2B). The slope of the line (seawater fish) in Figure 4.2A (bl.s^{-1}) was tested against a slope of unity (*i.e.* a line with $b = 0$), and against the slope of the freshwater chinook (dotted line). The analyses determined that the slope of the seawater chinook was significantly different from unity ($p < 0.001$, t-test), but not different from the slope of the freshwater chinook ($p > 0.05$, t-test). The slopes of the freshwater and seawater chinook in

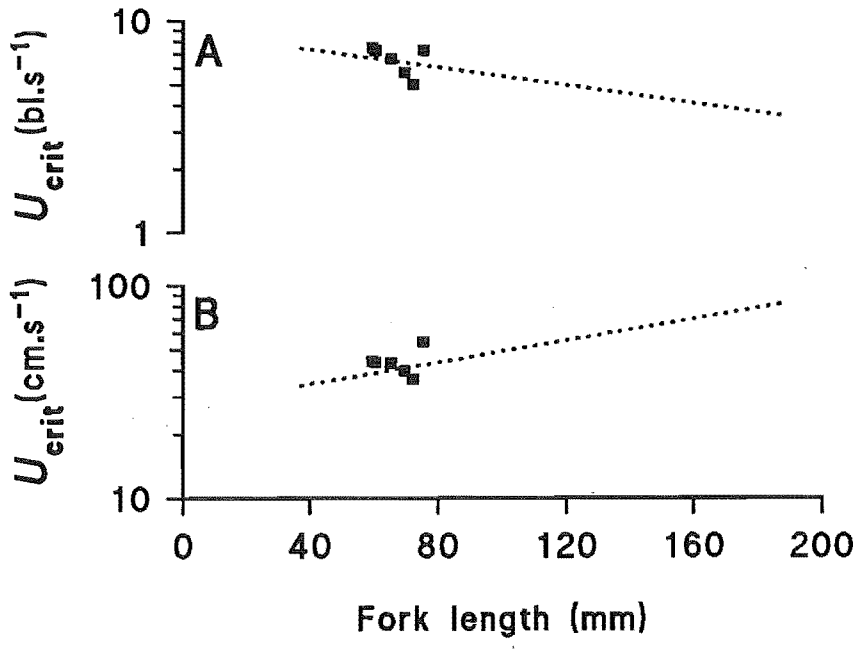


Figure 4.3 Individual measurements of the U_{crit} swimming performances of wild chinook salmon, expressed as relative speed (bl.s^{-1}, Figure 4.3A) and real speed (cm.s^{-1}, Figure 4.3B) against fork length (mm). Regression lines for untrained hatchery chinook salmon have been plotted for comparison (dotted line).

Figure 4.2B were not significantly different ($p>0.05$, *t*-test).

A one way analysis of variance comparing the U_{crits} of wild and untrained hatchery fish showed that there was no difference between the groups for either measurement of swimming performance ($p>0.05$ for both bl.s^{-1} and cm.s^{-1} U_{crit}, one way ANOVA), as shown in Figure 4.3A and Figure 4.3B. The wild fish that were subjected to U_{crit} tests had a mean fork length of 67.1±6.45 mm (mean±95%CL, $n=6$). The mean U_{crits} attained by these fish were 43.3±6.04 cm.s^{-1} and 6.5±0.99 bl.s^{-1}. The one way ANOVA compared the results for the wild fish with a group ($n=6$) of similar sized fish, picked at random from the untrained hatchery fish data set.

The sockeye data (Figure 4.4) were grouped into two sets, with mean fork lengths of 85.2±3.40 mm ($n=12$), and 123.2±9.22 mm ($n=9$). A two way analysis of variance compared the U_{crits} of the two groups of sockeye with those of similar sized untrained hatchery chinook (each cell in the two way analysis of variance test had nine ($n=9$) U_{crit} measurements, picked at random from all the possible 'smaller sockeye' and untrained chinook (*i.e.* over similar fork length) data sets). The analysis showed that both parameters of U_{crit} performance were significantly lower in the sockeye salmon ($p<0.01$ for both relative (bl.s^{-1}, Figure 4.4A) and real (cm.s^{-1}, Figure 4.4B) swimming speed; two way ANOVA). Some of the sockeye were not killed after measurement of U_{crit}. Instead these fish were returned to their rearing tank. Four sockeye died within 24 hours of the measurement of U_{crit}.

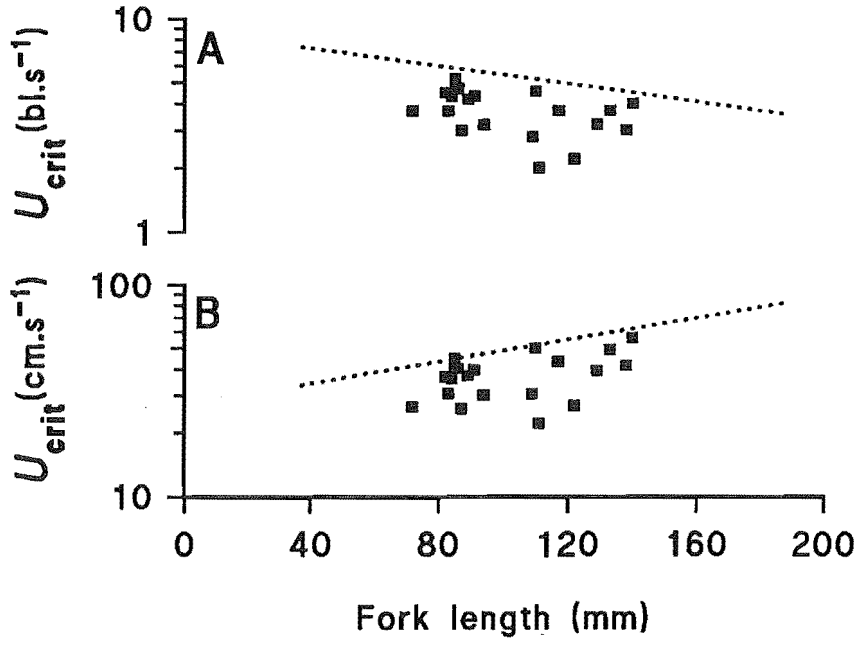


Figure 4.4 Individual measurements of the U_{crit} swimming performances of hatchery reared sockeye salmon expressed as relative speed, in body lengths per second (bl.s^{-1}, Figure 4.4A) and real speed (cm.s^{-1}, Figure 4.4B) against fork length (mm). Regression lines for untrained hatchery chinook have been plotted for comparison (dotted lines).

Physiological comparisons between 'resting' and U_{crit} fish

Physiological status of the fish immediately after the determination of U_{crit} was assessed using a number of parameters. Gill Na^+-K^+-ATPase enzyme activity, blood haematocrit, and the plasma variables, sodium, chloride and osmolality were measured. Data for untrained chinook subjected to U_{crit} analysis have been compared to the 'resting' levels in untrained chinook (Figures 4.5 and 4.6). The results from U_{crit} groups of trained chinook, and untrained, seawater resident chinook, were compared to resting levels in similarly treated, though 'untested' chinook salmon (Table 4.2). U_{crit} groups of untrained sockeye were compared to resting, untrained sockeye salmon (Table 4.2). Physiological measurements were not recorded from the wild chinook. The samples for each group was collected on the same day as that U_{crit} measurements were made.

Figure 4.5 (gill Na^+-K^+-ATPase activity and blood haematocrit) and Figure 4.6 (plasma osmolality, and plasma sodium and chloride concentration) compare physiological data between resting and U_{crit}, untrained hatchery chinook against day of the year. The data of season 2 (squares) and 3 (circles) have been pooled in both Figure 4.5 and Figure 4.6. Where possible, statistical significance between the untrained and trained fish has been compared using one way ANOVA, with significance ($p<0.05$) recorded by asterisks (*).

Although mean gill Na^+-K^+-ATPase activity (μmol P_i.mg protein^{-1}.h^{-1}) was consistently higher in fish that were subjected to a U_{crit} test, no significant difference was found between pairs of resting and U_{crit} groups in any experimental trial ($p>0.05$, one way ANOVA). In

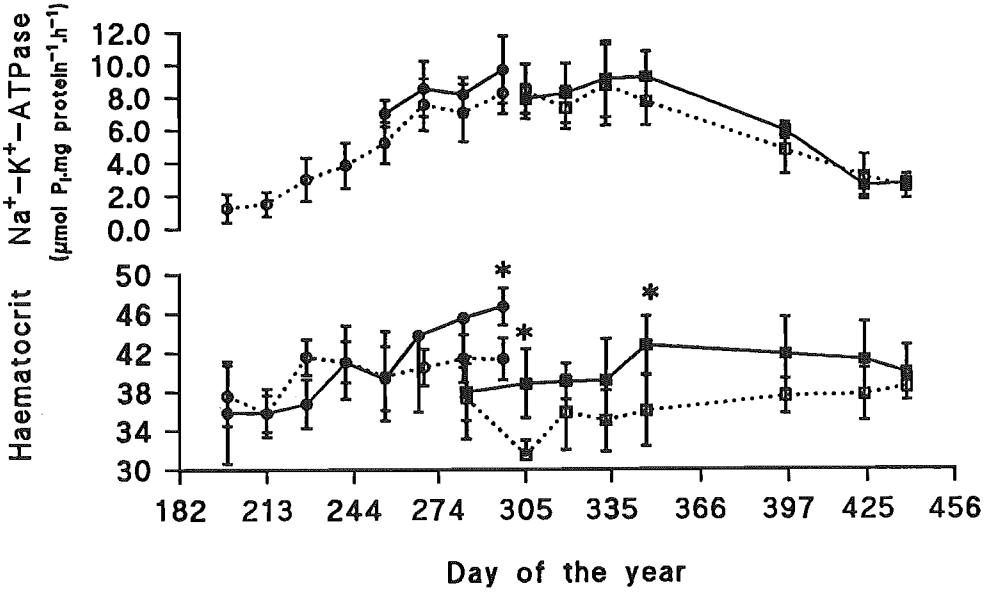


Figure 4.5 Comparison of gill Na^+-K^+-ATPase activity and blood haematocrit between resting untrained fish (open symbols, dotted lines) and untrained fish subjected to a U_{crit} test (solid symbols, solid lines) against day of the year. The numbers along the abscissa represent the first day of the month in a 365 day year. Because growing seasons over ran two calendar years, the first of January, February, March and April are represented as the 'day of that year plus 365' (*see* DATA HANDLING section and Figure 2.7 in CHAPTER 2). Data from season 2 and 3 have been pooled, with squares representing experimental trials from season 2, and circles, the trials from season 3. Data points indicate the mean $\pm 95\%$ confidence limits ($n=6$) for each group. Statistical significance between pairs of resting and U_{crit} fish groups is indicated by asterisks (*: $p<0.05$, one way ANOVA).

addition, a one way analysis of variance comparing the pooled data for resting and U_{crit} fish indicated that there was no difference in gill Na^+-K^+-ATPase activity between the groups ($p=0.22$, one way ANOVA).

The relationship between U_{crit} and blood haematocrit is complex. Overall (pooled data from both seasons), measurement of U_{crit} caused an increase in blood haematocrit ($p<0.001$, one way ANOVA). However, when the seasons were treated separately, haematocrit was only significantly higher in U_{crit} fish from season 2 ($p<0.0001$ for season 2, and $p=0.21$ for pooled trials of season 3, one way ANOVA). Each experimental pair of resting and U_{crit} group means were compared using one way analysis of variance, and significance has been indicated by asterisks (*) in Figure 4.5. Significance between the groups was only recorded during the period of parr-smolt transformation, although it was not observed in every trial.

The most striking observation from Figure 4.6 is the consistently lower plasma chloride concentration measured in U_{crit} fish. Where statistical comparisons were possible, chloride was lower ($p<0.05$, one way ANOVA) in all the trials, except one. The data for plasma sodium and osmolality are not as conclusive. However, both parameters were generally higher in U_{crit} fish. Additionally, in one of the trials where statistical analysis was possible, both plasma sodium and

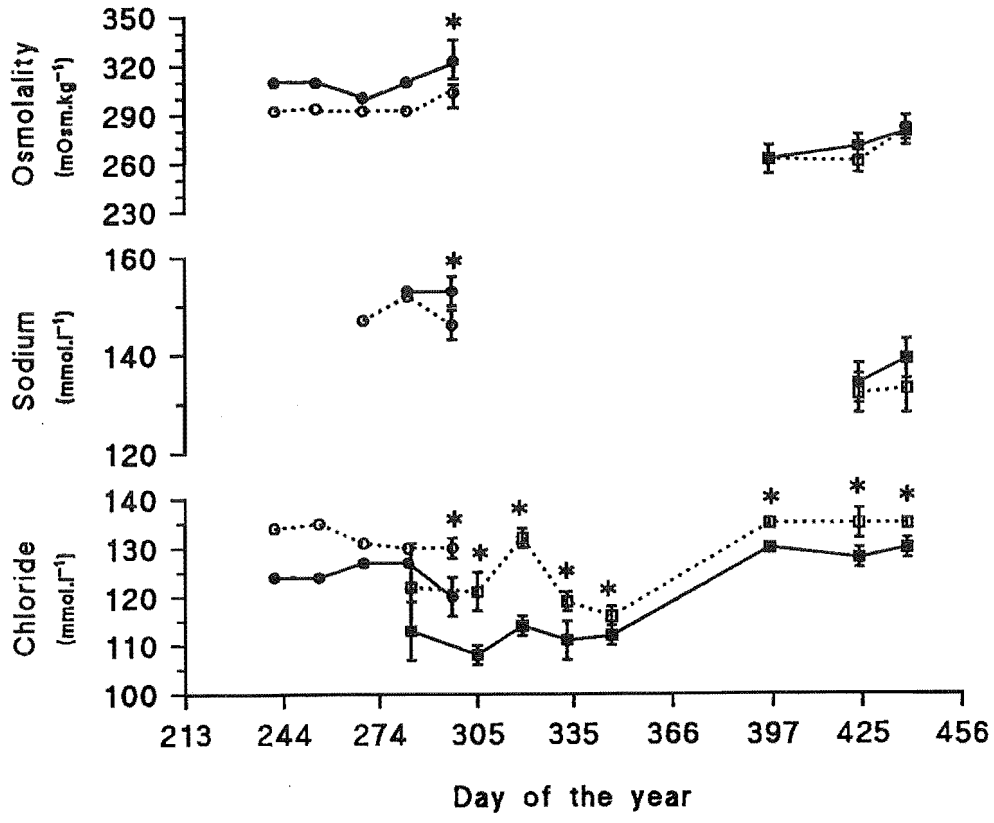


Figure 4.6 Comparison of plasma osmolality, and plasma concentrations of sodium and chloride between resting untrained fish (open symbols, dotted lines) and untrained fish subjected to a U_{crit} test (solid symbols, solid lines) against day of the year. Data from season 2 and 3 have been pooled, with squares representing experimental trials from season 2, and circles, the trials from season 3. Data points with error bars represent the mean ±95% confidence limits (n=6) for each group; data points without error bars indicate that the plasma of the six fish was pooled prior to analysis. Statistical significance between pairs of resting and U_{crit} fish groups is indicated by asterisks (*: $p<0.05$, one way ANOVA).

plasma osmolality were significantly higher in the U_{crit} fish ($p<0.05$, one way ANOVA) as indicated by the asterisks in Figure 4.6.

Table 4.2 presents data for physiological measurements (gill Na^+-K^+-ATPase activity, blood haematocrit, plasma osmolality, and plasma sodium and chloride concentration) recorded from groups of exercise trained, and seawater resident chinook, and sockeye salmon subjected to U_{crit} tests. Results for each group are compared with measurements recorded in 'resting' fish, *i.e.* fish that were not subjected to U_{crit} analysis.

The trends that were apparent between untrained, resting and U_{crit} chinook (Figures 4.5 and 4.6) are borne out between the treatment groups in Table 4.2. There was no difference in gill Na^+-K^+-ATPase activity between the groups in either experiment ($p>0.05$, one way ANOVA). Although mean blood haematocrit of U_{crit} fish was consistently higher in all three experiments, no significant difference was found at the 5% level of confidence, and only the haematocrit of exercise trained U_{crit} fish was higher at the 10% level (p=0.09, one way

ANOVA). Mean plasma osmolality was higher in U_{crit} fish across the experiments, but was only significantly greater in sockeye ($p<0.05$, one way ANOVA). The probability that differences existed between the values for resting and U_{crit} fish in the exercise trained and seawater resident experiments were almost significant, being $p=0.07$, and $p=0.10$ respectively (one way ANOVA). Although plasma sodium was not significantly different between the treatments in any of the experiments, it was almost significantly higher in the U_{crit} group of seawater resident fish ($p=0.06$, one way ANOVA). Plasma chloride was also greater ($p<0.05$) in the U_{crit} group of seawater resident fish. U_{crit} tests caused a lowering of plasma chloride in the other experiments (freshwater fish), but significance was only indicated in sockeye ($p<0.05$, one way ANOVA).

Table 4.2 Physiological data (gill Na^+-K^+-ATPase activity [μmol P_i.mg protein^{-1}.h^{-1}], blood haematocrit, plasma osmolality [mOsm.kg^{-1}], and plasma sodium and chloride concentration [mmol.l^{-1}]) for groups of fish subjected to U_{crit} tests compared with equivalent data for untested 'resting' fish. Data for exercise trained chinook, untrained seawater resident chinook, and untrained sockeye salmon are given. Gill Na^+-K^+-ATPase activity was not determined in the sockeye salmon. The number (n) of fish in each group was variable as indicated. Data are presented as the mean ±standard deviation. Statistical significance was determined by a one way analysis of variance between resting and U_{crit} fish for each physiological parameter, and is given by asterisks (*) when $p\leq0.05$. The paragraph mark ¶ is used when $0.10\geq p\geq0.05$.

Experiment	EXERCISE TRAINED CHINOOK		SEAWATER RESIDENT CHINOOK		SOCKEYE SALMON	
Treatment groups	Resting (n=3)	U_{crit} (n=3)	Resting (n=5)	U_{crit} (n=5)	Resting (n=5)	U_{crit} (n=5)
Na^+-K^+-ATPase	4.4±1.43	3.4±0.31	12.2±2.10	10.3±2.65	...	...
Haematocrit	37.2±2.52	43.7±4.52 ¶	39.1±2.83	40.1±2.90	36.0±1.22	39.1±4.30
Osmolality	300±6	309±3 ¶	324±16	346±20 ¶	301±10	323±11 *
Sodium	145±3	144±2	147±11	161±11 ¶	153±6	158±9
Chloride	125±8	120±4	132±1	142±9 *	130±4	122±5 *

DISCUSSION

The procedure used to measure critical swimming speed differed from previous methodology with respect to the magnitude of the water velocity increment, and the duration of each such increment (see Beamish, 1978 and Davison 1989). In this study, U_{crit} was determined with water velocity increments equivalent to one body (fork) length every five minutes. Brett (1964, 1967) advocated a set increment of 5 or 10 cm (regardless of fish length) every 60 minutes. However, the fish in Brett's work (in both the 1964 and 1967 papers, fork length ranged from 16-20 cm) were considerably larger than the size spectrum used in this study. A 10 cm increment for a 30-40 mm fork length fish was considered inappropriate, and was somewhat justified as the U_{crit}

measured in the smallest fish ('zip-up' fry) was approximately 25-30 $cm.s^{-1}$. As at least six fish were to be measured in one day, the interval between water velocity increments was restricted to five minutes. Therefore following the direction of Hoar and Randall (1978) to include increment duration and magnitude, the swimming performance results are described as *five minute, one body length per second* U_{crits}.

Farlinger and Beamish (1977) investigated the effect of varying both the magnitude of the water velocity increment and the duration between increments on critical swimming speed in largemouth bass (*Micropterus salmoides*). They found that increases of the interval between increments from 10 to 60 minutes effectively lowered U_{crit}, whereas increasing the magnitude of the increment from 5 to 25 $cm.s^{-1}$ caused critical swimming speed to reach a peak at around set increments of 10 $cm.s^{-1}$. Therefore, the measurement of U_{crits} made in this study (increment duration of five minutes, and increment magnitude of one body length (4-18 cm)) were likely to be fastest that 'maximal'. However, the fish were not in the 'post-absorptive' state, and therefore performance may have been reduced due to the specific dynamic action (ASDA, Beamish, 1974) of food, reducing the metabolic scope for activity (Jobling, 1981, 1983b).

Another assumption was taken that swimming performance did not vary on a diurnal basis, *i.e.* U_{crit} of any fish was independent of the time of day. Measurements of U_{crit} were made on individual fish to ensure that the physiological comparisons drawn were between 'resting' and exhausted (post U_{crit}) fish. The methodology behind the group measurement of U_{crit} records the critical swimming speed at the point where a certain percentage of the fish fatigue (Thomas *et al.*, 1964; Griffiths and Alderdice, 1972). Obviously, physiological analysis of fish treated in this way (*i.e.* group U_{crits}) would measure values from exhausted and non-exhausted fish, and would thus prevent true exhausted *versus* resting comparisons.

The results reported in this study are in general accordance with previous work. The fish that were exposed to the 'multiple' U_{crit} tests indicated that there was much individual variation between fish of a similar size, but that measurements of swimming performance were repeatable for individual fish. Fish used for repeated measurement of U_{crit} could be considered as having been 'conditioned'. Therefore subsequent determinations of U_{crit} could possibly show improvement with an increasing number of trials, and indeed slight increases in the mean U_{crit} were recorded in the second and third trials (Table 4.1), although they were not significant. Kolok (1992) reported similar results with U_{crit} work on largemouth bass. One fish used in the 'multiple' U_{crit} tests recorded a 'maximum' swimming speed of 9.6 $bl.s^{-1}$ (34.8 mm).

'Relative' critical swimming speed decreased exponentially with fork length in untrained chinook (Figure 4.1A). 'Zip-up' fry (mean fork length of 39 mm) attained critical swimming speeds of around 8 $bl.s^{-1}$, whereas the largest fish that was used had a U_{crit} of 3.7 $bl.s^{-1}$. Real speed U_{crit} increased with fork length (Figure 4.1B), as has been described previously in a number of species (Kerr, 1953; Bainbridge, 1960; Brett, 1964; Brett and Glass, 1973; Jones, 1971). The relationship between real speed U_{crit} and fork length appears to be asymptotic. Critical swimming speeds ('real' speed) of fry smaller than 40 mm fork length were markedly

lower than in all larger fish. Thomas and associates (1964) recorded swimming stamina ('performance rating') of juvenile, fall chinook fry and fingerlings, and found that the swimming performance (real speed) increased with size over the range 1-50 g (wet weight).

In a later paper (Thomas *et al.*, 1969), the same group investigated swimming performance in 'yolk-sac' fall chinook fry, and noted that at the time of complete yolk sac absorption, swimming performance decreased noticeably. They related this decrease in performance to the natural peak of fry outmigration from an artificial spawning channel. It is possible that the small 'zip-up' fry used in this study were at an equivalent stage of development as the 'yolk-sac' fry of used in the study by Thomas and his associates (1969). In this case the 'poor' swimming performance (in terms of real speed, $cm.s^{-1}$, only) recorded in these fish is indicative of metabolic changes occurring during yolk-sac absorption (It is necessarily important to clarify the units of swimming performance. Whilst the 'zip-up' fry swam 'poorly' in terms of real speed they achieved the fastest relative speed U_{crits}. Furthermore the fish that underwent the 'multiple' U_{crit} tests were smaller than the 'zip-up' fry mentioned above). Whether this reduced performance can be related to the period of maximum fry outmigration is another matter. The 'zip-up' fry of this study had a mean fork length of 39 mm, which is considerably larger than the size range of 33-37 mm for outmigrating chinook fry in Glenariffe Stream (Unwin, 1986).

Comparison of the U_{crit} data for untrained and exercise trained chinook (Figure 4.1) shows that exercise training had no effect on swimming performance. The exercise training regimes used (continuous or periodic swimming at 1-1.5 $bl.s^{-1}$) were apparently not enough to cause change. Both training regimes were aerobic, whereas the assessment of swimming performance demanded both aerobic and anaerobic sources of energy to be utilised. Improvements of swimming performance following training have been reported in the literature (Brett *et al.*, 1958; Hammond and Hickman, 1966; Greer-Walker and Pull, 1973; Farlinger and Beamish, 1978; Broughton *et al.*, 1980; Nahhas *et al.*, 1982b; Besner and Smith, 1983; Leon, 1986; Farrell *et al.*, 1991). There have also been studies that have not recorded enhanced U_{crit} following training (Brett, 1973; Farrell *et al.*, 1990). Improvements of swimming performance have been linked to a lower O_2 consumption rate, at any given swimming speed, and decreased blood titres of 'stress' hormones (Nahhas *et al.*, 1982b; Woodward and Smith, 1985).

An assessment of swimming stamina (time to fatigue at a constant water velocity) may have been a more applicable test of swimming performance. However a test of that nature was not attempted. Hochachka (1961) reported that the stamina of trained rainbow trout (*Oncorhynchus mykiss*) was almost double that of untrained fish after a training period of six months, and related the enhancement to the total store of metabolic fuel in the muscle, and the ability of the muscle to buffer the end products of anaerobic metabolism. Houlihan and Laurent (1987) reported similar results in much larger rainbow trout.

Untrained, seawater resident, hatchery fish (Figure 4.2) swam as well as the freshwater chinook provided the fish had made a successful transition to the marine environment. The fish that did not perform well in the U_{crit} tests (Figure 4.2) had exhibited poor growth or had not

grown at all since transfer to seawater (all seven fish were from season 2, *see* CHAPTER 6). Fully adapted, seawater resident fish (Figure 4.2), performed as well as their freshwater siblings in the U_{crit} tests. Although the gradient of the regression line through the seawater data for relative swimming speed ($bl.s^{-1}$, Figure 4.2A) was slightly less than that for the freshwater data, the lines were not different. The seawater regression line was different from a line with a gradient of zero, and therefore relative swimming speed was dependent on fork length. Although swimming performance of coho salmon (*Oncorhynchus kisutch*) transferred directly to seawater can be greatly reduced for up to four weeks post transfer, fish transferred at their optimal 'smolt status' do not show such a reduction in swimming performance (Flagg and Smith, 1982; Flagg *et al.*, 1983). The chinook used in this study had been resident in seawater for at least a week before their U_{crit} were measured, and only those fish that exhibited little or no growth had poor swimming abilities.

The wild chinook used in this study swam as well as hatchery reared chinook at the same size. Life in the natural environment had not conferred a greater swimming ability on the wild fish. Winz (1986, in Taylor and Foote, 1991) reported maximum differences of one $bl.s^{-1}$ in U_{crit} test between wild and domesticated rainbow trout (*Oncorhynchus mykiss*). He suggested that the poor swimming performance of hatchery fish may have been due to reduced selection pressure for swimming ability as a result of hatchery practices. Such a situation has also been recorded elsewhere (Duthie, 1987) for domesticated rainbow trout.

Because the wild fish were caught on their downstream migration at two or three months old, they could be considered as being in the 'smolt' stage of development. Therefore, that the swimming performance of the wild fish was not different from the hatchery fish adds further evidence that the swimming ability of juvenile chinook is not compromised during parr-smolt transformation. This is in contrast to research on juvenile coho (*Oncorhynchus kisutch*: Glova and McInerney, 1977; Flagg and Smith, 1982; Smith, 1982; Flagg *et al.*, 1983), and Atlantic salmon (*Salmo salar*: McCleave, 1978; Thorpe and Morgan, 1978b; Thorpe *et al.*, 1981; Virtanen and Forsman, 1987; Thorpe *et al.*, 1988). These workers believed that the downstream migration of smolts is essentially a passive process, not accomplished by active and directed swimming, and therefore would be facilitated by a reduction in swimming ability.

Raymond (1968, 1979) calculated downstream migration rates of juvenile chinook salmon and steelhead (*Oncorhynchus mykiss*) along the Snake River (a tributary of the Columbia River). He showed that displacement was 30-50% slower through impoundments (reservoir lakes) compared to travel time through free flowing portions of the river. In his summary, Raymond (1979) concluded that the time taken to complete the seaward migration had increased as a direct result of reservoir and hydroelectric dam construction. Smith (1982) used Raymond's (1979) data to argue that swimming ability of chinook smolts were reduced during the seaward migration. Johnson and Groot (1963) and Groot (1965) discussed seaward migrations of juvenile sockeye salmon through Grand Central Lake and Babine Lake in British Columbia. The seaward migration of sockeye smolts toward the lake outlet was accomplished by active and directed swimming (Groot, 1965).

Table 4.3 Records of the critical swimming speeds of sockeye salmon from this study, and those published in the scientific literature. Relative critical swimming speed ($bl.s^{-1}$) was calculated from the mean real critical swimming speed ($cm.s^{-1}$) and mean fork length data, and vice versa. Sources of the data are given below the table, the methodology regarding the magnitude and duration of each velocity increment differed between the studies.

Source	Mean fork length (cm)	U_{crit} ($cm.s^{-1}$)	U_{crit} ($bl.s^{-1}$)
Brett *et al.*, 1958	7.10	32.64	4.6
	13.92	46.32	3.3
Brett, 1964	18.8	76.8	4.1
Brett, 1967	16.2	65.8	4.1
Brett and Glass, 1973	16.2	59.0	3.7
Taylor and Foote, 1991*	7.77 ± 0.45^a	60.1 ± 0.52	7.7 ± 0.07
	7.75 ± 0.45^b	53.3 ± 0.53	6.9 ± 0.07
	9.14 ± 0.41^a	58.5 ± 0.56	6.4 ± 0.06
This study§	8.54 ± 5.46	35.6 ± 5.86	4.2 ± 0.66
	12.33 ± 1.23	39.8 ± 11.38	3.2 ± 0.84

* data are presented as mean $\pm$standard error ($n=50$).

a progeny of pure bred sockeye ♀ × sockeye ♂ crosses.

b progeny of pure bred kokanee ♀ × kokanee ♂ crosses.

§ data are presented as mean $\pm$95% confidence limits (small group, $n=12$; large group, $n=9$).

Sockeye had significantly slower critical swimming speeds than chinook over the size range where comparisons were possible (Figure 4.4). The behaviour of the sockeye was very placid in comparison to the chinook. Chinook devoured feed with a tremendous 'feeding frenzy' when feed was presented. Sockeye on the other hand, took feed in a more controlled, less aggressive manner. The swimming ability of juvenile sockeye salmon has been studied before; the results from these studies are given in Table 4.3. Direct comparison between the studies is misleading as the methodology (apparatus, and magnitude and duration of each water velocity increment) differed in the studies. Brett and co-workers undertook a number of studies on the swimming ability of sockeye salmon (Brett *et al.*, 1958; Brett, 1964, 1967; Brett and Glass, 1973). It is apparent from Table 4.3 that although much variation in U_{crit} exists both within and between different populations, and races of sockeye salmon, the data presented here are in good agreement with the findings of Brett and his associates (particularly Brett *et al.*, 1958). In a recent paper (Taylor and Foote, 1991), a comparison of the swimming ability of sockeye (anadromous) and kokanee (non-anadromous) indicated that the former race out performed the latter by about one body length per second (Table 4.3).

The sockeye in New Zealand were derived from an anadromous stock of the Fraser River (*see* CHAPTER 1; Scott, 1984; Hardy, 1983). However they have been voluntarily landlocked

ever since their introduction (Scott, 1984; McDowall, 1990b) - a period of some 90 years, and 25-30 generations. It has been shown that populations of fish that undertake extensive marine migrations have greater swimming stamina than fish that do not voyage as far whilst in the ocean (Tsuyuki and Williscroft, 1977; Besner and Smith, 1983; Taylor and McPhail, 1985c). The U_{crit} data for New Zealand sockeye fall at the lower end of the reported range (Table 4.3), and are much lower than recorded by Taylor and Foote (1991). A reduction in swimming ability may have occurred in the New Zealand sockeye as a result of their landlocked existence and the relatively short migrations that they undertake between spawning ground, and lake.

It was interesting that four of the sockeye subjected to measurement of U_{crit} died within 24 hours of the test (all four fish performed well in the U_{crit} test). This has been noted before by Wood and co-workers (1983) in rainbow trout forced to undertake severe (chased) exercise, and was proposed to have been caused by intracellular acidosis. Ferguson and Tufts (1992) also found that mortality increased in exercised rainbow trout exposed to a brief period of air exposure after the exercise period. These authors related that their findings had implications for 'catch-and-release' fisheries. The sockeye that died in this study all recovered from being anaesthetised for remeasurement after the U_{crit} test. However, once transferred back to their glass holding tanks, they generally remained at the bottom of the tank during 'recovery', and could not swim steadily, in a co-ordinated, controlled fashion.

The physiological data comparing resting levels with those in fish subjected to measurement of U_{crit}, indicate that changes arose as a result of the exhaustive swimming test (Figures 4.5 and 4.6, and Table 4.2). Elevated blood haematocrit has been recorded previously in fish subjected to exhaustive swimming tests (Milligan and Wood, 1987; Thomas *et al.*, 1987; Pearson and Stevens, 1991a,b; Wells and Weber, 1991; Butler *et al.*, 1992; Gallaugher *et al.*, 1992), the rise being attributed to splenic contraction (transfusion of cells), and erythrocyte swelling. Additionally, elevated blood haematocrit could be a non specific stress response to the U_{crit} test (the test acting as the stressor: Schreck, 1982b). Comparison of all the experimental trials indicated that haematocrit was frequently elevated in U_{crit} fish. However, haematocrit was only significantly greater within U_{crit} fish of each trial during the period of parr-smolt transformation, a stressful stage of development in the life history of salmon (Hoar, 1988). Virtanen and Forsman (1987), working on wild Atlantic salmon, found no difference in haematocrit between resting and exercised (3 $bl.s^{-1}$ for eight hours) parr, but greatly elevated levels in similarly treated smolts.

No difference in gill Na^+-K^+-ATPase activity was recorded during any of the experiments (Figure 4.5, and Table 4.2). Mean rates of activity were generally higher following the U_{crit} test in the untrained, freshwater fish (Figure 4.5), but there was no statistical difference ($p=0.22$, one way ANOVA). Why a U_{crit} test should increase gill Na^+-K^+-ATPase activity is uncertain. However, a similar result ($p<0.10$, Student's unpaired *t*-test) was found between resting and exercised (3 $bl.s^{-1}$ for eight hours) wild smolts of Atlantic salmon by Virtanen and Forsman (1987). The effect of sprint training on gill Na^+-K^+-ATPase activity would be worthy of investigation.

Blood plasma changes following a U_{crit} test markedly altered plasma chloride ion concentration (Figure 4.6, and Table 4.2). In freshwater experiments, U_{crit} tests tended to lower plasma chloride relative to the level in resting fish (chinook and sockeye), possibly through increased urine flow, or passive loss from the gills (Wood and Randall, 1978c). The large loss of chloride ions and relatively stable sodium levels would necessitate that some other anion (*e.g.* carbonate, or lactate) was elevated in the plasma to preserve electroneutrality. In contrast, U_{crit} tests involving seawater resident chinook increased plasma chloride concentration over resting levels. Haemoconcentration was likely to have occurred in the fish subjected to U_{crit} tests, as both plasma osmolality and sodium concentration also tended to be elevated in U_{crit} fish. The osmolality and sodium concentration data are in agreement with the finding that gill Na^+-K^+-ATPase activity was typically raised in freshwater U_{crit} fish. That all three plasma parameters were raised in the seawater resident fish (Table 4.2), is also in accord with the indication that gill Na^+-K^+-ATPase activity was slightly reduced during measurement of U_{crit}, and that increased (passive) ion diffusion may have occurred during U_{crit} tests.

Previous work done on rainbow trout and Atlantic salmon indicate variable results. Butler and associates (1991) found no change in either sodium or chloride ion concentration during their study. Wood and co-workers (1983) similarly found no changes in any of the plasma parameters during enforced burst swimming, although large increases were observed for several hours after the swimming bout. Wood and Randall (1973a,b) reported an overall increase in plasma sodium concentration following exercise that was attributable to a decrease in blood volume. The same authors indicated that urine flow increased markedly during periods of exercise (Wood and Randall, 1973c). Virtanen and Forsman (1987) reported decreases in plasma chloride concentration in wild Atlantic salmon smolts following enforced swimming at 3 $bl.s^{-1}$ for eight hours. Osmolality was also lower in exercised smolts, but there was no observable difference in osmolality between similarly treated parr. Additionally, Byrne and colleagues (1972) did not report changes in plasma osmolality, sodium or chloride ion concentration following exercise in Atlantic salmon (*Salmo salar*). However, all three parameters showed marked increases after exercise in their seawater resident fish.

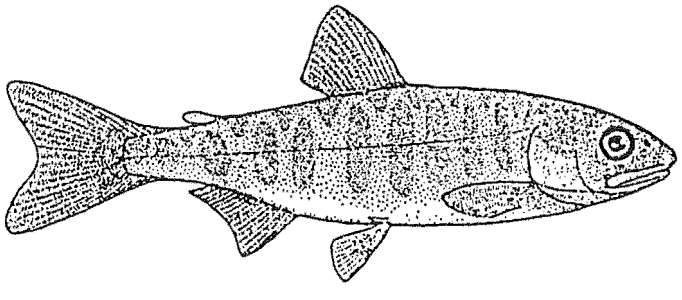
Finally, it was interesting to observe several swimming behaviours during the U_{crit} tests. Many of the fish showed a tendency to be 'unwilling' swimmers at the lower water velocities equivalent to 1-3 $bl.s^{-1}$, and much slower than their eventual critical swimming speed. This has not been reported before but has been noted previously in salmonids (Dr W Davison, personal communication, 1990). An explanation for this behaviour might lie in the musculature of the fish. Salmonids have essentially three muscle types, red (slow twitch), white (fast twitch) and pink (slow and fast twitch) fibres. The red muscle is powered by aerobic metabolism, whilst the white and pink from anaerobic sources. Red muscle is used for long duration, 'slow' swimming ('cruising'), the white for short duration, fast and burst swimming. Young salmonids do not naturally undertake steady undulatory swimming, they either avoid currents and hold station using non-body swimming (paired fins) or exhibit bouts of swimming behaviour interspersed frequent periods of acceleration and deceleration (Kerr, 1971; McNeish and Hatch,

1978). It may be that at these slower velocities, the water speed was too fast to be powered entirely by the red muscle, and yet too slow for the white muscle to be recruited. The size constraints of the swim-tunnel may have prevented the fish from using dart-and-drift type swimming at these slow velocities, leading to an abandonment of swimming.

Although dart-and-drift (burst-and-coast, Videler and Weihs, 1982) swimming has not (as far as I am aware) been recorded before in the salmonids, ram ventilation of the gills has been reported for rainbow trout (Steffensen, 1985) and sockeye salmon (Smith *et al.*, 1967). Both modifications of 'normal' swimming behaviour would confer energetic advantages (savings) as the fish reach the limit of their swimming performance.

CHAPTER FIVE

DEVELOPMENT OF A ROUTINE SEAWATER CHALLENGE TEST FOR UNDERYEARLING CHINOOK SALMON (*Oncorhynchus tshawytscha* Walbaum) IN NEW ZEALAND



CHAPTER FIVE

DEVELOPMENT OF A ROUTINE SEAWATER CHALLENGE TEST FOR UNDERYEARLING CHINOOK SALMON (*Oncorhynchus tshawytscha* Walbaum) IN NEW ZEALAND

INTRODUCTION

Propagation of anadromous salmonids in marine sea cages and through ocean ranching operations has become an important commercial venture in many temperate regions. Many fish farms are situated in outlying, economically depressed areas, and together with the associated processing businesses, they represent a major source of employment and income in those communities (Laird, 1989). Additionally, farming of salmonids epitomises a means of diversification to land owners, and can prove lucrative provided a quality product is cultivated. An essential element of both sea cage rearing and ocean ranching, is to minimize fish mortality during transfer and adaptation to the marine environment.

The ability of juvenile salmonids to successfully adapt to seawater is dependent upon the prior development of a 'smolt condition' with respect to osmoregulatory physiology (Hoar, 1976, 1988). Recognition of full smolt status is therefore pertinent for the timing of seawater transfer. Parr-smolt transformation involves a series of complex biological modifications occurring sequentially and in parallel with ontogenetic growth and development. Many of these parameters (such as gill and gut Na^+-K^+-ATPase activity, and gill, mitochondrial succinic dehydrogenase (SDH) activity, plasma hormone kinetics, integument silvering, and proximate analysis determination) may be measured (Hoar, 1976, 1988; Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980; Bern and Mahnken, 1982; Langdon, 1985; Thorpe *et al.*, 1985; Hansen *et al.*, 1989a). Techniques for assessing indicators of parr-smolt transformation vary from the simple observation of external body colouration, to complicated biochemical, physiological, and radiochemical determinations of numerous biological factors and parameters.

Morphological and colour changes associated with parr-smolt transformation of some salmonid species are particularly marked. These 'indicators' have largely been defined from observations of 'wild' (*i.e.* naturally spawned) fish, outmigrating from their natal rivers. However, intensive culture of salmonids in an environment far removed from nature can lead to desynchronisation of all the changes associated with parr-smolt transformation (Hoar, 1988). There have been many studies indicating that factors known to be necessary for smolt survival in seawater (body silvering, peak gill Na^+-K^+-ATPase activity and peak concentration of thyroxine $[T_4]_{pl}$ in the blood) do not predict maximum adult return - the overall statistic that determines whether ocean ranching and seawater farming ventures are economically viable (Ewing, Fustish *et al.*, 1980; Ewing and Birks, 1982; Ewing *et al.*, 1985). Release of hatchery fish at the same time, and at an equivalent size to the wild fish has often resulted in poor returns of adult fish. This is especially applicable to the pilot, ocean ranching feasibility experiments

carried out in this country (Unwin, 1985; Unwin *et al.*, 1989). From this work, it was apparent that releases of underyearling smolt (weighing less than 10 g) seldom yielded adult returns over 0.5% (a return of 2% is considered to be the 'bench mark' for economic viability of ocean ranching ventures). Interestingly the average size of underyearling, wild chinook smolts in New Zealand is approximately 60 mm fork length, corresponding to a wet weight of 1.93 g (data from Unwin (1986) for Glenariffe Stream fish).

A release of 35 g, hatchery yearling chinook in August 1979 produced a total adult return of 2.5%, which suggested that size and age, or both, at release had a significant influence on survival (Unwin, 1985). Since then, fish reared at Glenariffe for eventual release have been reared to 50 g or heavier, and are released as yearlings. This method of rearing fish for ocean ranching is in contrast to North America, where chinook are routinely released as '90 day smolts' (approximately 75-80 mm fork length, weighing 4-5 g; Childerhose and Trim, 1980). The reasons why the release of small underyearling fish show such poor returns as adults in New Zealand are debatable. Unlike their wild counterparts, the hatchery fish are thought to become disorientated at sea (McDowall, 1990b). Their small size, predator 'naivety', and the large number released at any one time have been suggested to result in massive mortalities to schools of kahawai (*Arripis trutta*), and other marine fish in the wash at the river mouths (Flain, 1981b). The 'carrying capacity' of the Canterbury Bight is also a possible limiting factor (McDowall, 1990b; Todd and Unwin, 1990; Unwin *et al.*, 1991).

The increase in hypo-osmoregulatory ability associated with parr-smolt transformation is a cyclical, circannual phenomenon (Eriksson and Lundqvist, 1982; Hoar, 1988). In some salmon species, the ability to tolerate seawater is possible within the first few days and weeks (pink and chum). The other anadromous species require varying periods of time in freshwater before they develop the osmoregulatory 'machinery' necessary for seawater tolerance and the development of a full smolt condition. It is thought that every species has a 'critical size for smolting' threshold (Elson, 1957; Parry, 1960; Clarke, 1982). Thus, a certain weight must be attained before the fish develop the physiological transformations characteristic of smolts, and thereby survive a transition to the marine environment. Clarke (1982) gave the critical body weight for chinook salmon as five grams.

Eriksson and Lundqvist (1982) showed in a 14 month experiment that Baltic salmon (*Salmo salar*) fed surplus rations, and kept under constant photoperiod (12L:12D) and temperature (11.0±0.5 °C) exhibited periodic fluctuations of high and low condition factor. In addition, parr- and smolt-like appearances similarly altered with fish condition. The period between 'smoltings' was approximately 10 months. Their study indicated that an endogenous rhythm exists within salmonids with respect to development of hypo-osmoregulatory ability and seawater survival, and that for parr-smolt transformation to proceed 'normally' at definite, predictable periods, it must be modulated by the environment. Photoperiod, water temperature, the lunar cycle and the rate of acquisition of surplus energy are all thought to influence the developmental processes associated with parr-smolt transformation at certain 'critical periods'.

Many other studies have reported the incidence of 'smolt-parr reversion', or

'desmoltification' in young smolts that were prevented from reaching the ocean by some means or other. The ability to survive a transfer to seawater is transient, and therefore 'the smolt' represents only a brief (recurring) period of development. It would appear that the 'environmental salinity' regulates whether the factors associated with the 'smolt condition' (seawater tolerance) are maintained, or whether the fish's physiology (*e.g.* patterns of gill Na^+-K^+-ATPase or mitochondrial SDH activity) returns to a state more consistent with freshwater and the 'parr condition' (Conte and Wagner, 1965; Zaugg and McLain, 1970, 1972; Mahnken, 1973; Zaugg and Wagner, 1973; Epstein *et al.*, 1980; Boeuf and Harache, 1982; Gorbman *et al.*, 1982; Koch, 1982; Langdon, 1985; Zaugg, 1989; Beckman and Zaugg, 1990).

A method to pre-determine the optimum time for transfer and seawater survival and growth is an integral aspect of salmon farming. In the late 1970's Clarke and co-workers began a series of studies that detailed a 'seawater challenge test' to assess the salt water tolerance of young salmonids (Clarke and Blackburn, 1977, 1978; Clarke, 1982; Blackburn and Clarke, 1987). These tests challenged the hypo-osmoregulatory capacity of fish transferred to seawater over a 24 hour period. Changes in plasma sodium concentration were monitored before and after transfer. The work showed that smolts were able to regulate their plasma sodium within a few days of transfer. Plasma sodium in parr increased and remained high for several days. Some parr died during the seawater transfer period (Clarke and Blackburn, 1977, 1978).

On-growing of chinook salmon in sea cages in New Zealand is normally accomplished by transfer of underyearling smolts during their first spring of life (austral spring; September-November). Seawater transfers at this time are only successful provided the fish have attained sufficient weight over the first 2-3 months post hatching, have undergone complete parr-smolt transformation, and are therefore smolts. Franklin (1989) investigated seawater survival in chinook (and sockeye) obtained from the source as this study. He related that plasma cortisol kinetics during the first 48 hours of seawater transfer could be used to evaluate likely long term survival. Additionally, he determined that plasma chloride concentration at 24-48 hours post transfer was a good indicator of seawater adaptation in both chinook and sockeye salmon (Franklin, 1989; Franklin *et al.*, 1992). Measurement of these physiological parameters require varying degrees of complicated, methodolgical 'laboratory' based operations and specialised equipment that are unlikely to be readily available or affordable to most commercial operators.

The principal aim of this study was to determine a simple, routine method for evaluating seawater tolerance in New Zealand chinook salmon, using a seawater challenge test. It was hoped that a test could be developed that would have application to the salmon farming industry. A 48 hour seawater challenge test was routinely used (in preference to the 24 hour challenges of Clarke and Blackburn (1977, 1978)) to assess the hypo-osmoregulatory ability of the young salmon for the following reasons. It was reasoned that if a fish was able to survive for 24 hours in seawater, then it had a likely chance to survive for a longer period. If however, any given fish was not fully adapted to a marine existence prior to transfer, then physiological changes that would have occurred after 24 hours would likely be the same, if not greater, after a further 24

hour period in seawater. In an attempt to prevent adding the complicating effect of handling and measurement stresses onto the stress of sudden transfer to seawater, a longer period between measurements was allowed.

Survival over the test period (and beyond) was correlated with several physiological parameters measured during the tests. Seawater tolerance was assessed in fry, fingerling, smolt, and post-smolt fish during growth and development over the first nine months of life (post hatching). Seawater survival of wild underyearling (0+) and yearling (1+) fish caught on their downstream migration during the natural period of parr-smolt transformation were also assessed. Growth and growth rates of surviving seawater transferred fish are dealt with in the next chapter (CHAPTER 6, *salinity, improved trophic opportunity and growth*).

MATERIALS AND METHODS

Fish stocks and initial husbandry

The fish were derived from crosses of three and four year old, sea-run fish returning to the Ministry of Agriculture and Fisheries' (MAF), Glenariffe Hatchery. Fertilised ova and the progeny were reared under natural photoperiod and standard hatchery practices. All fish taken from the hatchery for the seawater transfer experiments were underyearling (0+) chinook. Eleven seawater challenge tests using hatchery reared chinook were performed in each of two seasons (season 2, 1990-1991; season 3, 1991-1992).

Groups of wild (*i.e.* naturally spawned) 0+ and 1+ fish were also tested in season 3. These fish were caught on their downstream migration in the fry trap across Glenariffe stream. Age of the wild fish was assessed by appearance with the assistance of MAF Technician, Mr JRE Sykes. Only one sample of wild fish was taken for experimentation. The wild fish were collected by Mr Sykes during 15 to 23 November 1991, and were transported to the Zoology Department (hereafter the Department) on 23 November 1991.

On arrival at the Department, fish were transferred to holding tanks and allowed to recover from transportation stress for at least 60-70 hours. All tanks were supplied with fresh artesian water flowing at a rate that ensured complete water exchanges every hour or less. In addition, good aeration and circulation of the water were effected by gently bubbling compressed air through air stones. All tanks were covered with plastic netting to prevent fish escape. Fish were not fed during the recovery period, or during the 48 hours of the seawater challenge test. Fish weights therefore, were not biased by the weight of residual feed in the gut.

During season 2, the fish used in the last three seawater challenge tests were marked with an Alcian Blue dye solution. During season 3, all the fish were individually cold-branded. Fish were lightly anaesthetised prior to measurement and marking to reduce handling stresses, and to ensure good, clean, visible brands were applied. An assumption was made that neither method of marking would affect survival of the fish in seawater.

Forty-eight hour seawater challenge test

After the recovery period, the fish were individually measured and recovered in aerated freshwater for at least 30 minutes prior to seawater transfer. All transfers were performed between 10 a.m. and 12 noon. The fish were transferred directly into full strength seawater (≈30‰, 80 litre tanks) by dip netting.

The seawater system in the Department is a closed, recirculated system. Seawater was set to flow into each tank at a rate of 4-6 litres per minute. Whilst in the Department, the salmon were reared under a 12L:12D photoperiod. The seawater temperature varied slightly (13-16 °C, in line with the freshwater; 13-15 °C), during the experimental months, as did salinity (28-38‰). Salinity was far more stable on a day to day basis. In season 2, fish were transferred to 80 litre opaque plastic tanks. In season 3, transfers were to 80 litre glass tanks.

Fish were observed immediately after transfer, and thereafter every 30-60 minutes over the next 48 hours. During the twelve hours of 'night', dim light from the corridor running along side the Aquarium Room permitted the fish to be viewed without startling them. General fish behaviour was noted at each observation. Dead fish, fish that had lost their balance equilibrium, and/or lying on the bottom of the tank, unable to swim were removed, and the time of death noted. These fish were killed (if necessary) using a concentrated anaesthetic made up in seawater and dissected. All the fish that survived the 48 hour seawater challenge test were remeasured at 48 hours post transfer. After remeasurement the fish were allowed to recover in seawater, and were then returned to the seawater tank, and fed thereafter at least five times daily to assess subsequent growth rate (*see* CHAPTER 6).

During season 3, six fish from the total pool of fish to be transferred to seawater were killed on the morning of a seawater challenge test to record initial, freshwater, resting levels of various physiological parameters (termed the 'initial group'). Similarly, at the 48 hour remeasurement, a second group of six fish were killed for physiological analysis (termed the '48 hour group'), and provided an indication of the physiological status of the fish after 48 hours in seawater. Fish that died as a result of seawater transfer were collectively termed the 'dead' group. Observations of fish growth in seawater, after the 48 hour seawater challenge test, are effectively beyond the scope of this chapter. Chapter 6 describes seawater growth and growth rates of these surviving fish.

Fish sampling and dissection of 'initial' and '48 hour' groups

The group of '48 hour' fish (providing the fish survived transfer to seawater) were killed by overanaesthesia at the 48 hour remeasurement. The 'initial' fish group was killed with anaesthetic made up in freshwater, all seawater fish were placed into an anaesthetic made up in full strength seawater. Once measured, fish were placed on a cold glass plate and the tail was severed immediately posterior to the adipose and anal fins. Blood was collected from the caudal vasculature into ammonium heparinised micro capillaries, which were then plugged and centrifuged at 5000 *g* for six minutes. Blood haematocrit was measured. The plasma from the capillaries was transferred to plastic vials and snap frozen in liquid nitrogen. A sample of white

muscle was dissected from the tail stump and weighed. Similarly the viscera were dissected from the carcass and weighed. These samples were then dried to constant weight. The gills were removed, rinsed in homogenising buffer (Johnson *et al.*, 1977), placed into plastic vials, and snap frozen in liquid nitrogen. All samples were stored at −80 °C until analysis.

Physiological analysis

Blood plasma was analysed for sodium and chloride ion concentration ($mmol.l^{-1}$) and osmolality ($mOsm.kg^{-1}$). Sodium concentration was analysed in a Varian Techtron 1200 Absorption Spectrophotometer. Chloride concentration was determined using a Radiometer CMT 10 chloride titrator. A Wescor Inc. 5100C vapour pressure osmometer was used to determine plasma osmolality. Plasma was pooled, as necessary, prior to analysis, to facilitate that all three determinations were measured for each experimental group. Activity of the enzyme Na^+-K^+-ATPase was measured in the gill epithelia, using previously described methods (Johnson *et al.*, 1977; Langdon *et al.*, 1984; Franklin, 1989).

Calculation of morphological indices and actual and percentage water loss

Fulton's condition factor, $100 \times W/L^3$ (where W is the gram weight, and L is fork length in centimetres), was calculated for each fish at every measurement. Percentage water content of the white muscle and viscera were calculated. Actual and percentage changes of fork length, weight, and condition factor were calculated for individual fish from the measurements made at the beginning and at the 48 hour period in seawater. For the seawater challenges (of season 2) in which the fish were not marked by dye or brands, the 'initial' measurements were ranked from smallest to largest on the basis of their fork length. The lengths at the 48 hour remeasurement (or death) were similarly ranked, and the percent changes of length, weight, and condition factor were calculated for each fish. Fork length was used to rank the data as it was the least variable measurement over the 48 hour period.

Statistical analysis

Results are presented as individual measurements, or as the mean ±standard deviation, or ±95% confidence limits. Statistical significance was assessed using one way analyses of variance (one way ANOVA). Significance was recorded when $p \leq 0.05$. Some of the data are presented in a graphical form with 'day of the year' represented along the abscissa. The numbers on such axes represent the first day of the month in a 365 day year (*see* DATA HANDLING section and Figure 2.7 in CHAPTER 2).

RESULTS

Fish behaviour during seawater challenge tests with 100% mortality

Upon transfer to full strength seawater fish normally started active swimming in the tank,

probably as a result of becoming positively buoyant. Ventilatory rates were higher than they were prior to measurement, probably as due to the handling and their increased activity. Ventilation (and seawater drinking in response to dehydration?; Usher *et al.*, 1988) remained high in these fish until death. Within two hours the fish had generally ceased active swimming and either held position in the water column or 'rested' on a tripod of fins (pectoral fins and the caudal fin) at the bottom of the tank. As the test proceeded more fish tended to favour 'resting' at the bottom of the tank. Swimming became increasingly less co-ordinated and ineffective - despite normal body undulation, directed thrust was not effected. A total loss of balance equilibrium and orientation preceded death, assessed as a lack of ventilatory movements. The skin colour darkened appreciably during the 48 hour test, with fish becoming dark grey and black in appearance. The smallest fish (mean fork length of 33.27 mm, mean weight of 0.242 g) died within 3½ hours of transfer. Overall survival time increased with fish size during the seasons. During the period of parr-smolt transformation survival was high (if not total) and then decreased. The largest fish that were transferred (mean fork length of 168 mm, and mean weight of 54.20 g) started to die within 25 hours of transfer, and all ten died within the 48 hour seawater challenge test.

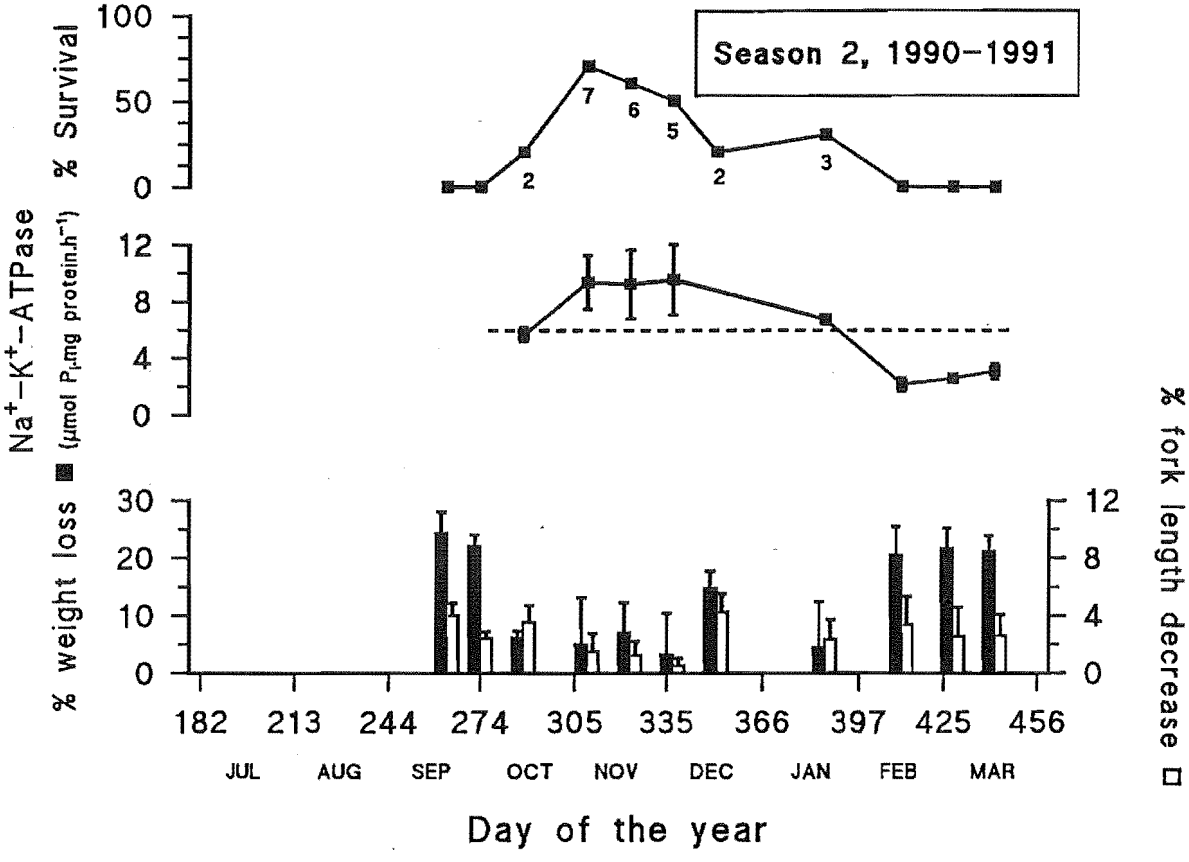
Fish behaviour during seawater challenge tests with 100% survival

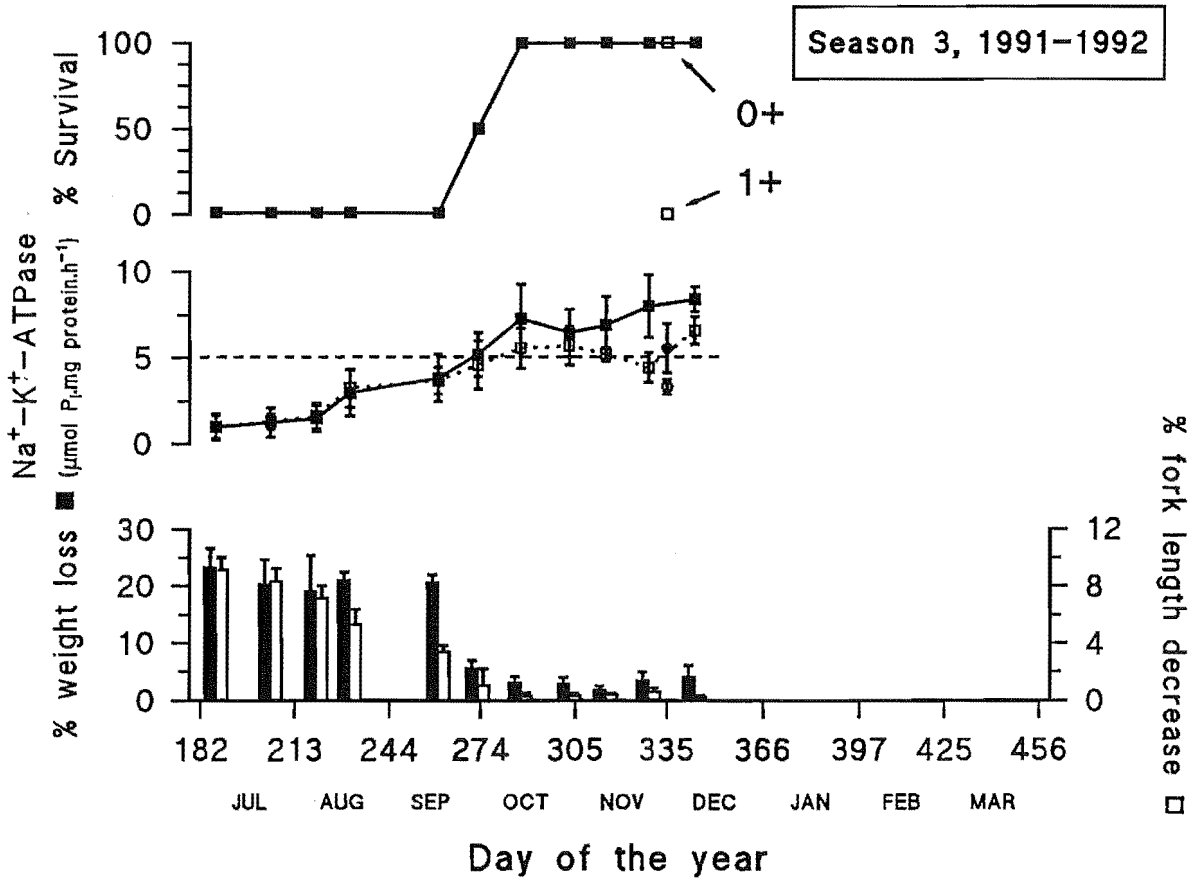
Upon transfer these fish behaved in much the same way as their siblings that eventually died. In short, increased activity was noted with active swimming and higher ventilatory rates than were recorded prior to transfer. In the main however, these fish did not maintain elevated ventilatory rates for the entire duration of the 48 hour seawater challenge test. These fish repeatedly swapped 'resting periods' at the bottom of the tank with co-ordinated swimming behaviour and steady position holding in the water column. Body colouration remained fairly constant, darkening slightly, but certainly not to the extent observed in those fish that failed to adapt to seawater.

Comparison of unsuccessful and successful transfers to seawater

Due to overlapping sampling dates, the data for the seawater challenge test from season 2 and season 3 have been treated separately as shown in Figure 5.1 (season 2) and Figure 5.2 (season 3). Each figure presents percentage survival, gill Na^+-K^+-ATPase activity, and losses of fork length and wet weight against time (day of the year). Percentage survival was calculated from the number of fish alive after the 48 hour seawater challenge period. When all fish died during the challenge test, the losses of length and weight were calculated from the dead fish. When fish survived the transfer to seawater the data for length and weight loss were calculated from the surviving fish only. Similarly, gill Na^+-K^+-ATPase activities in Figure 5.1 (season 2) were determined from dead fish when seawater transfers were unsuccessful, and from alive fish in those transfers when fish survived.

In Figure 5.2 (season 3), the gill Na^+-K^+-ATPase activities were calculated from the six fish in each of the 'initial' and '48 hour' groups. In both Figure 5.1 and Figure 5.2 data for


Season 2, 1990–1991
% Survival
100
50
0
7
6
5
2
2
3
Na+–K+–ATPase ■ (µmol Pi.mg protein.h-1)
12
8
4
0
% weight loss
30
20
10
0
% fork length decrease □
12
8
4
0
182 213 244 274 305 335 366 397 425 456
JUL AUG SEP OCT NOV DEC JAN FEB MAR
Day of the year


Season 3, 1991–1992
% Survival
100
50
0
0+
1+
Na+–K+–ATPase ■ (µmol Pi.mg protein.h-1)
10
5
0
% weight loss
30
20
10
0
% fork length decrease □
12
8
4
0
182 213 244 274 305 335 366 397 425 456
JUL AUG SEP OCT NOV DEC JAN FEB MAR
Day of the year

length and weight losses, and gill Na^+-K^+-ATPase activities have been presented as the mean ±standard deviation. Ten fish were transferred during each seawater challenge test of season 2, and there were six fish in each of the hatchery reared 'initial' and '48 hour' groups of season 3. There were five fish in each of the 'initial' and '48 hour' groups for the 0+ wild smolts. Six 1+ wild smolts were transferred to seawater.

From Figures 5.1 and 5.2, it is apparent that the ability to survive a transfer to seawater is transient. Survival was only observed during the months of October, November, and December (austral spring, and early summer). Size was an important factor in seawater survival, the smallest group to survive transfer being 64.59±2.12 mm (fork length, mean±95%CL) and 2.56±0.27 g (wet weight). No fish weighing less than two grams survived a transfer to seawater.

The percent survival, and mean values for percent length and weight loss, were correlated to mean values for gill Na^+-K^+-ATPase activity recorded from each transfer (Figure 5.3). Percentage survival was strongly correlated with gill Na^+-K^+-ATPase activity (r^2=0.689, n=19). The percentage loss of length and weight were also strongly negatively correlated with gill Na^+-K^+-ATPase activity (r^2=0.695, n=19; and r^2=0.795, n=19; respectively). Each parameter correlation with gill Na^+-K^+-ATPase was highly significant ($p<0.0001$). From the results presented here (Figure 5.3) it is apparent that gill Na^+-K^+-ATPase activities below 4 or 5 μmol P_i.mg protein^{-1}.h^{-1} ('crude' gill homogenates) were associated with a lack of seawater survival.

Weight loss in fish that failed the seawater challenge test was around 20-25% of the initial weight, irrespective of fish size. Surviving fish lost less than 5-10% of their body weight during the 48 hour seawater challenge test (Figures 5.1, 5.2, and 5.3). Furthermore, all fish that survived the seawater challenge test lost, on average, less than 1% of their fork length during the first 48 hours in seawater. Some (30%) of these fish did not show decreases in fork length, and indeed small (<2%) increases were observed, although the mean values for the groups indicated consistently that there tended to be slight decreases in fork length overall. In contrast, the fork length of all the fish that died during the seawater challenge tests had decreased substantially (Figures 5.1, 5.2, and 5.3). During the course of the study, a number of 'freshwater control' transfers were carried out. Six fish were measured and then transferred to a freshwater tank, at the same time that sibling fish were transferred to salt water for the seawater challenge test. These fish were remeasured at 48 hours 'post-transfer'. Although both decreases of mean group length (<1%) and weight (<2%) were observed during these 'control' tests, they were modest. Surviving, seawater transferred fish tended to lose slightly more weight than the fish kept in freshwater.

All the underyearling (0+, n=10) wild smolts survived the seawater challenge. Percentage loss of length and weight were 0.37±0.28%, and 3.43±2.09% respectively. The yearling (1+, n=6) wild fish did not survive in seawater. Two of the yearlings died within the 48 hours of the seawater challenge test itself, and the other four died within the next 48 hours. The two fish that died within 48 hours had lost 17.86% and 21.26% of their body weight, and

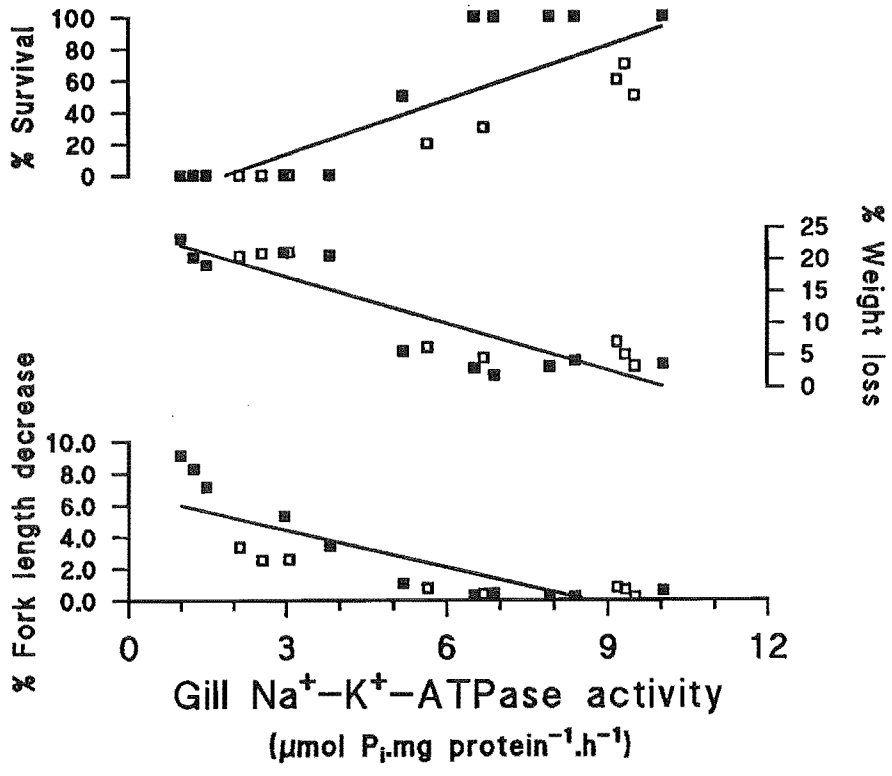


Figure 5.3 Correlations of percentage survival, percentage weight loss, and percentage fork length loss for underyearling chinook salmon following 48 hour seawater challenge tests with mean group, gill Na^+-K^+-ATPase activity. Open symbols represent the seawater challenge tests of season 2, and solid symbols those of season 3. Data for season 2 are from 'dead' or 'surviving' groups, whereas data for season 3 are from 'dead' or '48 hour' groups.

4.51% and 5.72% of their fork length. The four fish that survived to the remeasurement had lost at least 2% of their fork length, and 10% of their body weight. For the purpose of this analysis, these yearling fish were considered as having failed the seawater challenge test, as it could be *predicted* from their weight and length losses that they would not survive indefinitely, hence the 0% survival point in Figure 5.2. Furthermore, at the time of death, these four remaining fish had lost 7.07±2.62%, and 20.62±3.65% of their initial length and weight respectively, and had not fed. Interestingly, one yearling fish that was kept in freshwater during the period of the seawater challenge test also died within a week of capture.

Gill Na^+-K^+-ATPase activities increased some 20-25% during the successful seawater challenge tests involving hatchery chinook (Figure 5.2). The wild, underyearling (0+) chinook exhibited a 40% increase in gill Na^+-K^+-ATPase activity. In contrast, fish that failed to survive in seawater showed little or no increase in gill Na^+-K^+-ATPase activity at the time of death (Figure 5.2). It is possible however, as that some of the fish may have been dead for 30 minutes or more prior to dissection, that enzyme denaturation may have occurred, thereby reducing the 'apparent' gill Na^+-K^+-ATPase activity.

The percentage water content of the white muscle and viscera of 'dead' fish decreased

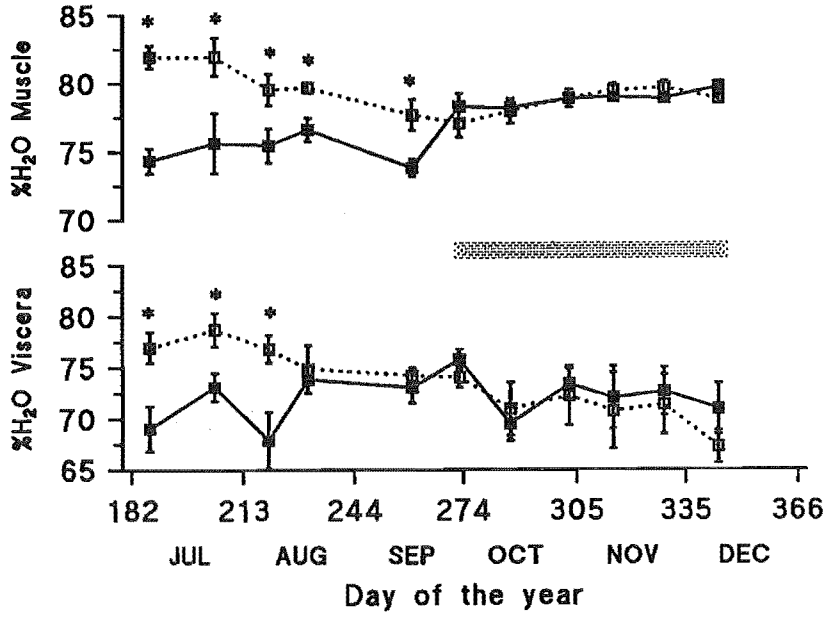


Figure 5.4 Comparison of the percentage water content of white muscle and viscera of freshwater 'initial' groups (open symbols, dotted line) and seawater transferred '48 hour' groups (solid symbols, solid line) fish against time (day of the year). The shaded box indicates the period during which fish survived the transfer to seawater (*i.e.* duration of parr-smolt transformation). Data points during that period compare tissue percentage water content of the 'initial' and '48 hour' groups. Data points before the period of parr-smolt transformation compare values from 'initial' and 'dead' fish. Data are given as the sample mean $\pm 95\%$ confidence limits ($n=6$ for 'initial' and '48 hour' fish, and $n=12$ for 'dead' fish), and are from the seawater challenge tests of season 3 only. Asterisks (*) indicate a significant difference ($p<0.05$, one way ANOVA) between 'initial' and 'dead' fish groups within each trial of the seawater challenge test.

significantly ($p<0.05$, one way ANOVA) during the unsuccessful trials of the seawater challenge test (Figure 5.4). Water content of the white muscle and viscera of 'zip-up' fry decreased by 9.3, and 10.3% respectively. When the fish started to survive the seawater challenge tests (*i.e.* during the period of parr-smolt transformation), the percentage water content of these tissues showed very little change after 48 hours in seawater (Figure 5.4). Indeed, the mean water content of the muscle and viscera were occasionally higher (relative differences of 0.5-2.0% for each tissue type) in the '48 hour' group. No significant differences ($p>0.05$, one way ANOVA) were found between the 'initial' and '48 hour' groups in the trials where there was 100% survival (Figure 5.4).

In conjunction with the decrease in water content of the tissues in 'dead' fish, blood haematocrit and the plasma variables of sodium and chloride ion concentration and osmolality increased. Although 'dead' fish (fish that died as a result of seawater transfer) had higher mean blood haematocrit than 'initial' fish, it was frequently very difficult to obtain blood from the 'dead' fish (such fish had lost 20-25% of their body weight). The blood from such fish was exceedingly viscous and would not readily flow into the micro capillaries. Values for

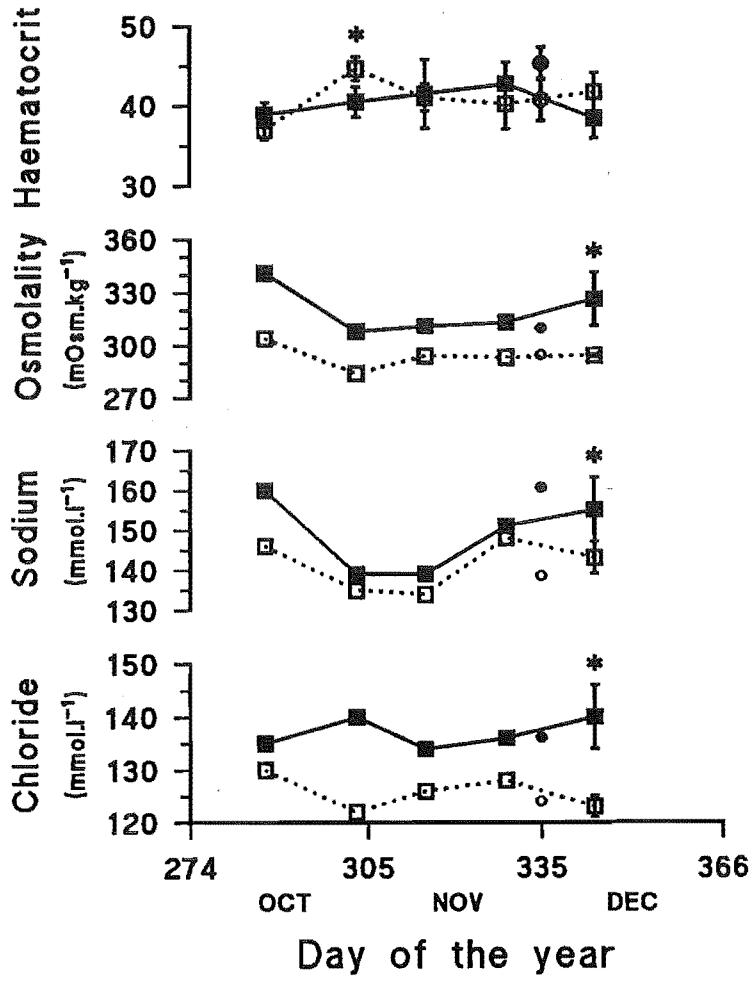


Figure 5.5 Comparison of haematocrit, plasma osmolality, and plasma sodium and chloride concentrations between the 'initial' and '48 hour' sample groups against time (day of the year). The data are from season 3, and are only from those seawater challenge tests that resulted in 100% survival of the fish (*see* Figure 5.2). The 'initial' groups are represented by open symbols and dotted lines, and the '48 hour' groups by solid symbols and solid lines. Hatchery reared fish (n=6) are given by squares, and the wild 0+ chinook (n=5) by circles. Haematocrit data are presented as mean ±95% confidence limits. Plasma was pooled prior to analysis in all the trials except the last, and therefore the plasma variable data are presented as the sample mean for four of the trials (and for the wild chinook), and as the mean ±95% confidence limits for the data from the last trial. Asterisks (*) indicate a significant difference (p<0.05, one way ANOVA) between the groups.

haematocrit of 'dead' fish were always higher than from the initial fish, and often accounted for more than 50% of the whole blood volume.

As a consequence of the high blood viscosity, only limited plasma was able to be analysed from the seawater challenge tests that quickly caused 100% mortality of the fish. Of the few plasma samples analysed, it was obvious that the hypo-osmoregulatory systems of the fish were dysfunctional or insufficient to meet the demands of the marine environment. Pooled plasma from the 'dead' fish (n=12) of the sixth seawater challenge test of season 3, had a

plasma concentration of chloride at 215 $mmol.l^{-1}$ (compared to the 'initial' freshwater mean of 131 $mmol.l^{-1}$), and sodium at 225 $mmol.l^{-1}$ (cf., 147 $mmol.l^{-1}$). Plasma osmolality was also greatly elevated at 467 $mOsm.kg^{-1}$ (cf., 293 $mOsm.kg^{-1}$). The fish of that particular seawater challenge had lost, on average, $21.21 \pm 2.56\%$ of their body weight (mean$\pm$95%CL), and $5.31 \pm 1.09\%$ of their fork length at the point of death.

During successful seawater challenge tests, the transfer to seawater caused slight increases (<20%) of all three plasma variables at 48 hours post-transfer. Figure 5.5 presents haematocrit and blood plasma data for the five seawater challenge tests that produced 100% survival of the hatchery chinook, and the successful seawater challenge with the underyearling, wild fish (*see* Figure 5.2). The plasma was pooled in all of these trials except the last hatchery transfer, and therefore statistical comparisons between all of the trials were not possible.

Blood haematocrits of 'initial' and '48 hour' fish during the period of parr-smolt transformation were variable, with no discernable trend. Haematocrit values for the '48 hour' seawater fish were never as high as those recorded from fish that died during the seawater challenge tests, and were not significantly different ($p > 0.05$, one way ANOVA) from those observed in the 'initial' fish in any of the trials that resulted in 100% survival. Clear differences were visible in the data for the plasma variables. Although statistical analysis was only possible on the data from the last trial, seawater transfer consistently effected increases of up to 20% above the 'initial' value for plasma osmolality, and plasma concentrations of sodium and chloride (Figure 5.5). In the one trial that permitted statistical analysis, all three plasma variables were significantly higher ($p < 0.05$, one way ANOVA) in the '48 hour' group. All three plasma variables remained higher than the 'initial' freshwater values whilst the fish were resident in seawater (*see* CHAPTER 6). The values recorded for the underyearling (1+) wild chinook are in general accordance with the hatchery fish data. Blood was not able to be collected from the yearling (1+) wild chinook. All these fish ($n=6$) died within 96 hours of transfer to seawater.

DISCUSSION

The ability of underyearling chinook salmon to withstand a transfer to seawater was readily determined from the 48 hour seawater challenge tests undertaken in this study. Previous accounts of similar tests have used seawater challenges of a 24 hour duration (Clarke and Blackburn, 1977, 1978; Clarke, 1982; Blackburn and Clarke, 1987; Zaugg and Beckman, 1990). These studies commonly used the measurement of a plasma variable (typically sodium concentration) as the determinant of hypo-osmoregulatory ability and seawater tolerance in the young salmon. Zaugg and Beckman (1990) however, working on yearling coho salmon (*Oncorhynchus kisutch*), correlated length and weight losses during a 24 hour seawater transfer period, with gill Na^+-K^+-ATPase activity.

From the results it is evident that chinook died very quickly (as early as two hours after transfer) prior to developing the hypo-osmoregulatory mechanisms necessary for adaptation to

the salt water environment. Fish size and gill Na^+-K^+-ATPase activity were important factors regarding the ability of young chinook to tolerate seawater, with the larger fish of any group (trials that resulted in 100% mortality) tending to outlive the smaller fish. Increased fish size of 'post-smolts' (after the period of parr-smolt transformation during season 2, Figure 5.1) enabled some fish to survive beyond the first 24 hours in seawater, although most died before the end of the 48 hours of the challenge test. Elevated activity of the gill Na^+-K^+-ATPase enzyme conferred a greater seawater tolerance in the fish. Seawater survival was not observed unless the enzyme activity was approximately 4 or 5 μmol P_i.mg protein^{-1}.h^{-1} or greater (using crude gill-extract homogenates).

In short, of all the trials that caused 100% mortality within the 48 hour period of the seawater challenge test, only 20% of the fish survived for longer than the first 24 hours. Furthermore, in the trials with partial survival (Figures 5.1 and 5.2), of all the fish that died as a result of seawater transfer, 69% did so within the first 24 hours, 17% within the next 24 hours, and 14% within the next 48 hours. All the fish that died as a result of seawater transfer, did not survive beyond 96 hours post transfer. Franklin (1989) found a similar result in his study of seawater adaptation in New Zealand chinook salmon. In contrast, other workers have described that other species of salmon become moribund as 'stunts' (Clarke and Nagahama, 1977), or 'parr-revertants' (Mahnken, 1973) if they are transferred to seawater at the wrong time with respect to the development of hypo-osmoregulatory ability. The ultimate fate of such fish is not necessarily death. However, the growth of such fish is poor in comparison to sibling fish that made a fully successful transfer to seawater (Otto, 1971; Mahnken, 1973; Clarke and Nagahama, 1977; Bern, 1978; Woo *et al.*, 1978; Folmar *et al.*, 1982; Gorbman *et al.*, 1982; Mahnken *et al.*, 1982).

Gill Na^+-K^+-ATPase activity has previously been used as the most reliable indicator of hypo-osmoregulatory ability in juvenile chinook salmon (Zaugg and McLain, 1972; Johnson *et al.*, 1977; Ewing and Birks, 1982; Franklin, 1989; Zaugg and Beckman, 1990). The results of this study do agree with the earlier work, as all fish that had an enzyme activity level less than 4-5 μmol P_i.mg protein^{-1}.h^{-1} failed to survive the seawater challenge test. The levels for gill Na^+-K^+-ATPase activity presented here are similar to those of other workers who used crude gill homogenates in the enzyme analysis (Johnson *et al.*, 1977; Franklin, 1989). Assays that use partially and wholly purified homogenates tend to produce enzyme activities that are much higher (Zaugg, 1982a). Transfer to seawater caused a rapid and substantial increase in gill Na^+-K^+-ATPase activity during the seawater challenge tests. Such an effect has been reported many times previously (*see* reviews by Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980; Langdon, 1985; Hoar, 1988), although the rise has generally taken longer to occur (3-5 days post transfer). Franklin (1989) did not report such an abrupt rise in gill Na^+-K^+-ATPase activity in his studies of chinook from the same stock.

There are three theories to explain the further rise of gill Na^+-K^+-ATPase activity of fish transferred to seawater (Wedemeyer *et al.*, 1980). Briefly, the first theory proposes an isozyme system, with two functional alleles for gill Na^+-K^+-ATPase, one for freshwater and the other

for seawater. The second suggests that the presence of seawater causes a conformational change of the gill epithelium and the unmasking of existing enzyme sites. The third relates that the presence of seawater causes a stimulation in the rate of *de novo* synthesis of the enzyme units. Towle and associates (1976) have cast doubt on the first theory, as they were unable to discern isozyme-like protein bands from the gills of the euryhaline blue crab, *Callinectes sapidus*. The same group (Towle *et al.*, 1977) did find that binding of ouabain (a specific inhibitor of Na^+-K^+-ATPase) increased with external salinity in the killifish, *Fundulus heteroclitus*, adding credence to the second theory - at least for euryhaline fish. Many papers on salmonid research give support to the third theory (Giles and Vanstone, 1976a; Dickhoff *et al.*, 1977; Folmar and Dickhoff, 1978, 1979).

A recent paper (Beckman and Zaugg, 1990) has provided evidence for the first theory. Actinomycin-D, a known inhibitor of messenger RNA (mRNA) synthesis of saltwater adapted eels, *Anguilla anguilla*, caused significant decreases in gill Na^+-K^+-ATPase activities in seawater resident fish, but no change in freshwater resident fish. The decline in the seawater fish was presumably due to degradation of existing enzyme units, without their replacement due to the blocking action of actinomycin-D on *de novo* synthesis. The authors went on to suggest that the rise in gill Na^+-K^+-ATPase activity over the period of parr-smolt transformation that has been observed in gill homogenates, reflects the increased production of non-functional (in freshwater) seawater isozyme units within the chloride cells of the gills. These isozyme units only become *functional* upon entry to the marine environment following activation/stimulation by some 'salt water factor'(Beckman and Zaugg, 1990).

Zaugg and Beckman (1990) used the phrase 'saltwater-induced decreases in length and weight' to describe the changes observed in their study of yearling coho salmon (*Oncorhynchus kisutch*). Whilst the phrase appropriately describes the large reductions of both length and weight observed in the 'dead' (non-surviving) fish of this study, it is perhaps not so apt for the fish that survived the transfer to seawater. Although fish transferred to seawater for 48 hours without feed lost slightly more weight than sibling fish held in freshwater, little or no decrease in length was observed in either of the groups over the same time. However, the differences between surviving and 'dead' fish were quite conspicuous (Figures 5.1, 5.2, and 5.3). Moreover, it was possible to *predict* whether fish would continue to survive beyond the period of the seawater challenge test by calculating the percentage decreases of length and weight at the 48 hour remeasurement, and from observation of colouration and general behaviour. All the fish, except one, that had lost more than 10% of their body weight after 48 hours in seawater died within the next 48 hours. The solitary fish that contradicts this statement was transferred in late November 1992. This fish lost almost 12% of its body weight during the seawater challenge test, and became quite dark as a result of transfer. In spite of this, the fish survived, grew well, and upon dissection was discovered to be undergoing precocious maturation of the testes (*see* CHAPTER 7).

An apparent disparity between the survival data of season 2 and 3 may be explained by the quality of the seawater in the closed, recirculated seawater system in the Departmental

Aquarium (Figures 5.1 and 5.2). Prior to season 3, there was not an effective particle or biological filter in operation (a large 200 litre tank, full of the common mussel, *Perna canaliculus* served as the only seawater filter). High nitrite, nitrate, and ammonia concentrations were in the seawater at all times despite routine and repeated flushing with 'fresh' seawater. Between season 2 and season 3, the entire seawater system was overhauled and reconditioned, and a particle filter was installed. The system was refilled with 'fresh' seawater, and as a result the quality of the water was much improved. Another difference between the seawater challenge tests of season 2 and season 3 was that the season 2 tests were carried out by transferring fish to dark blue, plastic tanks, whilst those of season 3 were to clear, glass tanks. Tank colour has been reported to affect parr-smolt transformation, and reduce seawater survival (Kato, 1972, given in Stefansson and Hansen, 1989; Stefansson and Hansen, 1989). Notwithstanding water quality and tank colour, the calendar period during which some or all of the young salmon 'smolts' survived the seawater challenge was similar between the two seasons (Figures 5.1 and 5.2).

All the post-mortem, and physiological data are in agreement with the general outcome of the seawater challenge test. In Chapter 3 (*see* Figure 3.6), it was shown that the percentage water content of the white muscle and the viscera varied with growth and development, and time. Statistical comparison of percent water content between the pre-transfer, freshwater ('initial') fish and the seawater transferred, 'dead' and/or '48 hour' fish are therefore necessarily restricted to within each trial of the seawater challenge test. From Figure 5.4 it is obvious that the weight loss of 'dead' fish was attributable to severe dehydration of the tissues. In surviving fish however, no differences between the 'initial' and '48 hour' groups were recorded in percent water content of either the white muscle or the viscera.

Dehydration was not limited to the intracellular environment, as indicated by the haematocrit and plasma data. Non-surviving, 'dead' fish had exceedingly viscous blood that produced high measurements for haematocrit. The concentrations of the plasma variables were also notably elevated, and outwith their natural 'physiological ranges'. Weisbart (1968) studied the development of osmotic and ionic regulation in embryos, alevins, and fry of the five North American Pacific salmon. The concentrations of the three plasma parameters recorded here for the 'dead' fish are similar to those for Weisbart's 'LD_{50} chinook'. Transfer to seawater generally causes transient increases of plasma ionic concentrations in young salmonids (Komourdjian *et al.*, 1976a; Blackburn and Clarke, 1987; Hoar, 1988, and references cited therein). Elevated plasma concentrations of sodium and chloride, and plasma osmolality were apparent in the fish of the '48 hour' groups (Figure 5.5).

The hypo-osmoregulatory ability of young anadromous chinook increases with growth and development (Wagner *et al.*, 1969). The increase in the time to death may be explained in part by the decrease in the surface area to volume ratio of larger fish. At the point of death however, it would appear that there is little difference in weight loss between dead fish, irrespective of size. Clarke (1982) argues that a 'critical size for smolting' exists in all anadromous salmonids, and that provided the physiological transformations for a life in salt

water have been completed at the time of transfer, young salmon will successfully adapt to, and grow in the marine environment. In his review, Clarke (1982) indicated that underyearling chinook with a body weight of five grams had attained optimal hypo-osmoregulatory ability. Clarke and Shelbourn (1985) detailed that 'fry' (1.50 g, body weight) from a population of Canadian fall chinook, survived and grew well in seawater. The optimum size for sodium regulation and growth, at seawater transfer however, was found to be when the fish were 5-6 g at transfer. The fish used in this study did not survive a transfer to seawater until they had reached at least 2.0-2.5 g in weight. However, this minimum size for seawater tolerance is somewhat smaller than reported by Franklin (1989), which was seven grams. Gill Na^+-K^+-ATPase activities were not at their highest in the small, 2.0-2.5 g chinook (Figure 5.2), and therefore it is likely that the whilst hypo-osmoregulatory ability of those fish was sufficient to survive a transfer to seawater, it was not 'maximal' relative to the ability of older and bigger fish transferred at a later date. This finding is in general accordance with the work of Clarke and Shelbourn (1985).

Seawater survival of the wild chinook provided interesting results. Both underyearling (0+) and yearling (1+) smolts were subjected to the seawater challenges. All the 1+ smolts died within 96 hours of transfer. The 0+ smolts on the other hand fared as well as their hatchery reared counterparts at the same time, with 100% survival. Furthermore, the physiological data obtained from the wild fish were equivalent to those from the hatchery fish (Figure 5.5). With certain reservations as to the validity of the experiment regarding 'stress' in the wild fish, the data for seawater survival of the wild fish are, on the face of it, in general agreement with the belief that 0+ smolts contribute about 75% or more of the wild run of adults (McDowall, 1990b; Mr F Lucas, personal communication, 1992). However, survival of the 1+ smolts should have been better than that of the 0+ smolts to agree this the current theory and the age structure of the spawning population data (McDowall, 1990b).

In any given year, the number of outmigrating 0+ smolts far exceed the number of 1+ smolts (Unwin, 1986). The subsequent survival of the 1+ smolts is thought to be far higher than that of the 0+ smolts. The product of relative survival and sheer number of migrating fish produces the 3:1 proportion of 0+ smolts over 1+ smolts observed in the spawning population. From the results presented here, it would appear that the survival of the 1+ smolts was very poor. It is however unlikely that the 1+ smolts would migrate downstream without due cause and purpose. The wild fish were caught during the natural period of parr-smolt transformation of New Zealand chinook (Unwin, 1986; McDowall, 1990b). Perhaps the 1+ fish were thoroughly stressed by the experience of capture, transportation and handling, and being held within the unnatural environs of the Department, and were therefore unable to survive the additional stress of the sudden transfer to seawater. I feel that this assumption is justified by the unaccountable death of the one yearling smolt that was kept in freshwater whilst its siblings underwent a seawater challenge test.

The New Zealand chinook salmon was established from the progeny of fall run, ocean-type, Californian stocks (*see* CHAPTER 1). The high survival of the 0+ smolts agrees

well with the natural history of their ancestral stocks (Kjelson *et al.*, 1982), and the normal life history pattern of ocean-type chinook salmon (Healey, 1983, 1991). This study of wild smolt survival during a seawater challenge test was, as far as I am aware, the first of its kind using wild smolts in this country.

The high correlation between gill Na^+-K^+-ATPase activity and fish survival, and decreases of fork length and body weight following seawater transfer, indicates that the 48 hour seawater challenge test described here may well be a valid means of determining the hypo-osmoregulatory ability and capacity of young chinook salmon. The test is relatively simple, does not require the measurement of physiological parameters, and would not necessarily require the use of individually marked fish. Although in this study the branding irons were cooled in liquid nitrogen, branding irons cooled in acetone and dry ice have provided an effective means of fish marking (Everest and Edmundson, 1967). In addition, 'hot' brands (branding irons in boiling water) have also been used successfully in salmonid research (Mighell, 1969). Smith (1973) found that both hot and cold branding of juvenile salmonids was equally successful, and therefore any given method could be chosen on the basis of ease and availability.

Decreases of either length or weight would serve as adequate indicators of hypo-osmoregulatory ability and full smolt condition. An outlay of expenditure would be required for both; Vernier callipers or a fairly sensitive (accurate to 0.01 g or less), digital balance. Additionally, a small seawater aquarium or tank facility, or the ability to quickly and easily transport relatively small numbers of smolts to a 'testing pen' at the sea cage location is a necessary consideration for this test to have practical application. If decrease in length were used preferentially to loss of weight as the indicator, a period of starvation prior to the test would not be necessary, as weight lost through defecation would be of no consequence.

There is no reason why the seawater challenge test outlined here could not be performed on larger, yearling, hatchery reared chinook to determine hypo-osmoregulatory ability of 'smolts' prior to their release or transfer to on-growing seawater facilities. The early work by MAF has shown that ocean ranching businesses are unlikely to gain from the release of small, underyearling chinook (Unwin 1985; Unwin *et al.*, 1989). However, farmers that on-grow their salmon in sea-cages may well benefit from using a seawater challenge test during the spring months (October, November and December) once their chinook have attained a body weight of five grams or greater. The method of determining seawater tolerance of young salmon outlined here could be applied to the other species of salmon that are commercially reared in seawater net-pen facilities.

OUTLINE OF THE SEAWATER CHALLENGE TEST PROTOCOL

Obvious limitations exist regarding the applicability of a seawater challenge test. Ideally facilities should be available for holding a relatively large number (up to 200) of fish, both in fresh- and seawater. If body weight were to be measured, the fish would have to be held in

freshwater for 48 hours to permit the emptying of the gut. If fork length were to be measured, this holding period would not be necessary. Fish could be branded (hot or cold brands), although this is not an absolute requirement. At all times, the fish should be handled as little and as gently as possible.

The fish should be lightly anaesthesised and individually measured for fork length (fish could be weighed in groups provided each fish was adequately 'dried' with a non abrasive, soft, absorbent tissue paper). A record of each 'initial' measurement should be kept. A recovery period of at least half an hour is recommended to ensure complete recovery from anaesthesia.

Fish were dip netted into the seawater facility in his study to minimise dilution of the seawater. The method of transfer would depend on the seawater facility into which the fish were to be placed. It would be necessary, if the fish were to be placed into a sea cage, to reduce the volume available to the fish to ensure that capture for the 48 hour remeasurement was easily expedited.

Mortality during the 48 hour test should be monitored and dead fish removed (provided this was possible without unduly stressing the fish). Remeasurement at 48 hours should be accomplished as with the 'initial' measurements. Return surviving fish to seawater testing facility.

Calculation of the salt water induced losses of length and/or weight are as follows

$$\frac{INITIAL\ FkL\ -\ 48\ HOUR\ FkL}{INITIAL\ FkL} \times 100$$

or

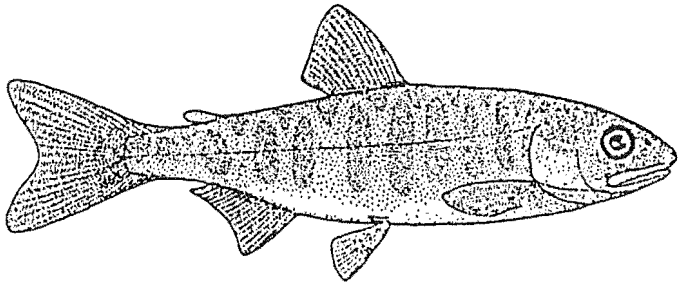
$$\frac{INITIAL\ Wt\ -\ 48\ HOUR\ Wt}{INITIAL\ Wt} \times 100$$

i.e. subtract the '48 hour' measurement from the 'initial' measurement and divide this figure by the 'initial' measurement. Multiply this result by 100 to convert to a percentage.

Losses of less than 2% of fork length and 5% of wet weight were strongly correlated with seawater survival, and should therefore be used as the yardstick values for assessing likely seawater survival. However, transfer of underyearling salmon during their first period of parr-smolt transformation indicated whether survival of the salmon was likely purely on a percentage survival basis. Furthermore, a 24 hour seawater challenge test would probably be sufficient for these fish, as death within the first 24 hour period of the seawater challenge was high in those fish. The rationale for using a 48 hour test was borne out with the larger yearling fish. Due to their larger size, a number of such fish survived beyond the first 24 hour period in seawater, although most died within the 48 hours of the seawater challenge test. Saltwater induced changes of length and/or weight could predict likely seawater survival thereafter. A 24 hour seawater challenge may well be suitable therefore for the determination of seawater survival, however such methodology would obviously require experimentation and verification.

CHAPTER SIX

THE EFFECT OF SALINITY AND IMPROVED TROPHIC OPPORTUNITY ON THE GROWTH OF UNDERYEARLING CHINOOK SALMON (*Oncorhynchus tshawytscha* Walbaum)



CHAPTER SIX

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INTRODUCTION

Salmonids grow faster in the ocean than in the freshwater environment. This generalisation is made with the assumption that one is comparing free ranging, 'wild' (*i.e.* naturally spawned) fish that migrated to sea as smolts (anadromous), with sibling fish that remained in freshwater (landlocked). Growth rate is enhanced in the oceanic environment due to a number of factors, although principally it is increased by greater feeding opportunity. Salmonids to most people are large, silver bodied, sea-run fish, and this large size is naturally attained from considerable somatic growth at sea. Evidence for good growth in the marine phase comes from many sources.

Juvenile anadromous species of salmon migrate to sea as smolts, approximately 28-35 mm (fork length) in pink salmon, *Oncorhynchus gorbuscha* (Heard, 1991), to around 100-150 mm in Atlantic salmon, *Salmo salar*, smolts (Thorpe *et al.*, 1981). Adult salmon return to spawn after varying periods of time at sea, having grown to approximately 30-100 cm in length (depending on the species, and the number of years at sea). Atlantic salmon smolts are at least one year old when they migrate to sea, and weigh approximately 15-50 g (Wedemeyer *et al.*, 1980; McCormick and Saunders, 1987). They may return to spawn 14-15 months later as 'grilse', after one sea-winter, weighing about 1.5-2.5 kg (Thorpe, 1980; McCormick and Saunders, 1987). Growth and growth rate therefore, are considerably greater in the marine phase. Pacific salmon also exhibit this trend, most noticeably in the sockeye (*Oncorhynchus nerka*). Sockeye salmon exist as three distinct forms (with respect to their natural life cycle): the anadromous sockeye; the non-anadromous kokanee; and the non-anadromous residuals (non-migrating offspring of anadromous sockeye; Ricker, 1940). Of the three forms, sockeye (up to 65 cm) are the largest at full adult size, followed by the kokanee and the residuals (around 18-30 cm; Ricker, 1938, 1940; Burgner, 1991).

Comparison of growth rates of chinook salmon (*Oncorhynchus tshawytscha*) in fresh and seawater are possible from data on the wild stocks that are found in New Zealand. Yearling smolts (roughly 15 months old) have a fork length of approximately 60-100 mm (Field-Dodgson, 1985; Unwin, 1986), whereas yearling fish caught at sea are nearly double this size (Finlay, 1972). Final adult size of spawning fish also differs between sea-run and voluntarily landlocked chinook. Size data for year classes of sea-run and landlocked chinook spawners were provided by Flain (1972a). Although he did not report the source of the landlocked fish in the paper, they were from the Lake Coleridge population (Mr M Flain, personal communication, 1993). Flain's size at spawning data are compiled into Table 6.1 for age classes of 2, 3, 4, and 5 year old fish.

The sea-run fish data are from wild fish returning to spawn in Glenariffe Stream (five year old chinook are extremely rare in New Zealand, and rarely constitute more than 1% of the spawning population, Flain, 1982). Although most (>95%, Flain, 1982) two year old sea-run fish are males, both males and females are represented in the two year old landlocked fish. From Table 6.1 it is evident that sea-run chinook grow to a larger size as two year old fish than landlocked chinook attain after five years of growth. Moreover, the size difference between the sea-run and landlocked age classes increases with age, such that 5 year old, sea-run chinook can be up to five times heavier and approximately double the length of five year old landlocked chinook.

Table 6.1 Spawning size of sea-run and landlocked chinook salmon at different ages (for populations of New Zealand chinook). Data are given as the mean size for fork length, and body weight (males and females combined) in each age class. Sea-run fish data are from spawners returning to Glenariffe Stream, whereas landlocked fish are from Lake Coleridge (after Flain, 1972a; Mr M Flain, personal communication, 1993).

Spawning Age and stock	Fork Length (cm)	Body Weight (kg)
2 year old, sea-run	58	2.3
2 year old, landlocked	36	0.68
3 year old, sea-run	76	5
3 year old, landlocked	46	1.1
4 year old, sea-run	89	6.8
4 year old, landlocked	51	1.5
5 year old, sea-run	102	10
5 year old, landlocked	58	1.8

It was originally thought that salmon must migrate to sea to realise their growth potential and large size, and that continued rearing in freshwater not only inhibited growth (Hoar, 1939; Power, 1959), but could cause fish mortality (Koch *et al.*, 1959). It is now known that if smolts are retained in freshwater, they 'desmoltify', *i.e.* they revert (morphologically, behaviourally, and physiologically) to the parr stage (Houston, 1957, 1961; Saunders, 1960; Evropeitseva, 1962; Conte and Wagner, 1965; Hoar, 1976, 1988; Zaugg and McLain, 1970, 1972; Zaugg and Wagner, 1973; Lasserre *et al.*, 1978; Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980; Koch, 1982; Johnston, 1983; Langdon, 1985). In short therefore, the physiology of a smolt is pre-adapted to life in a marine environment, whereas that of a parr is suited to freshwater. Environmental cues can alter the course of developmental growth in juvenile salmonids and confer the physiological ability to survive a transfer to seawater during critical periods ('time windows', Boeuf and Harache, 1982). If for some reason such fish are prevented from migrating to the sea, the osmoregulatory physiology returns to levels that are adaptive for continued life in freshwater.

It was thought that the difference regarding the size disparity of adult fish must be due to the inherent growth rate of seawater adapted fish being considerably higher than in sibling fish in freshwater (Koch *et al.*, 1959; Hoar, 1965; Saunders and Henderson, 1969). In addition, some workers suggested that maximum growth rate of salmonids would be observed in brackish, isotonic (isosmotic) water, where the osmotic potential of the fish and the environment are equal. It was argued that in such an environment, the metabolic cost of maintaining ionic homeostasis would be minimal, and therefore greater energy could be directed toward somatic growth. Conflicting results have been published regarding salinity and its effect on salmonid growth (Bullivant, 1958, 1961; Canagaratnam, 1959; Rao, 1968; Saunders and Henderson, 1969; Otto, 1971; Kepshire and McNeil, 1972; Shaw *et al.*, 1975b; Gjedrem and Gunnes, 1978; Brett, 1979; Brett and Groves, 1979; Clarke *et al.*, 1981; Nahhas *et al.*, 1982a; McKay and Gjerde, 1985; McCormick *et al.*, 1989; Morgan and Iwama, 1991).

Biological systems have functional temperature optima with regard to growth rate (Brett, 1979). It follows therefore that environmental temperatures either side of optimum will reduce growth. Water temperatures below 5 °C can arrest growth in salmonids (Murray and McPhail, 1988; Beacham and Murray, 1990; Murray *et al.*, 1990; but *see* Higgins and Talbot, 1985), and temperatures above 25 °C are considered as lethal to salmonids (Brett, 1979). Warm water (1-5 °C above 'normal') can accelerate the growth rate of juvenile salmonids (Donaldson and Brannon, 1975; Brannon *et al.*, 1982; Saxton *et al.*, 1983; Kazakov *et al.*, 1988; Soivio *et al.*, 1988), advance the period of parr-smolt transformation (Zaugg *et al.*, 1972; Zaugg and McLain, 1976), and thereby reduce the time spent in the freshwater phase of juvenile development (Hoar, 1988). However, such elevated and 'artificial' water temperatures also hasten a decrease in the duration of the parr-smolt transformation period by simultaneously facilitating the rate of 'smolt-parr reversion' or 'desmoltification' (Zaugg *et al.*, 1972; Novotny, 1975; Zaugg and McLain, 1976; Donaldson and Brannon, 1975; Clarke and Nagahama, 1977; Clarke *et al.*, 1981; Clarke and Shelbourn, 1985; Soivio *et al.*, 1988). Recent work has shown that accelerated rearing in warm water over winter months may be detrimental to the subsequent survival of Atlantic salmon in seawater (Dickhoff *et al.*, 1989). In contrast, Kasahara and co-workers (1989) have reported that accelerated rearing of masu salmon (*Oncorhynchus masou*) leads to a more synchronous parr-smolt transformation in that species and therefore the possibility of improved seawater performance upon release.

Intra-specific competition in the juvenile stages of fish development is well documented (Weatherley and Gill, 1987). Commercial salmon hatcheries rear juvenile fish at stocking densities that are far in excess of those observed in the natural environment. High stocking densities can lead to serious decreases in water quality (low oxygen content and coincident higher nitrogen content and increased ammonia concentration in the water), and increased intra-specific competition for feed and 'space', if un-monitored. Both factors can reduce individual growth rate in various species of commercially reared salmonids, increase fish mortality rates, and affect the development of 'indices of smoltification' and thereby seawater survival (Reftsie and Kittelsen, 1976; Reftsie, 1977; Schreck, 1982b; Jobling, 1985; Schreck *et*

al., 1985; Vijayan and Leatherland, 1988; Holm *et al.*, 1990). However, a number of other studies have not found a 'density effect' with regard to growth, survival, and seawater performance (Carl, 1984; Soderberg and Meade, 1987; Fagerlund *et al.*, 1987; Kjartansson *et al.*, 1988).

It was not possible to separate, and therefore individually measure, the direct effect of water temperature and stocking density on the growth rate of chinook salmon using the facilities available for this study. However the fish used in this research would have experienced elevated water temperature, decreased stocking density, and therefore probably increased feeding potential upon transfer to the Zoology Department (hereafter the Department). Therefore, the effect of 'improved trophic opportunity' on growth rate of young chinook was examined.

The purpose of this study was to assess the effects of various environmental conditions on the growth rate of underyearling chinook salmon. The effect of an 'improved freshwater environment' on growth was assessed by comparing the growth of fish at a commercial hatchery with sibling fish reared in the laboratory. The effect of long term (up to seven months), accelerated rearing on growth to the 'post-smolt' stage was investigated in fish that had been reared in the warmer water of the laboratory (14-15 °C), as opposed to the cooler water of the Glenariffe hatchery (7-13 °C). Growth and growth rates in seawater were investigated in those fish that survived a transfer to the marine environment (*see* CHAPTER 5). Laboratory reared, seawater resident fish were reared in the same way as the freshwater 'accelerated' fish. Seawater growth rates were therefore compared to those of sibling fish reared in the laboratory.

MATERIALS AND METHODS

Fish stocks

The fish used in this study were obtained from the Ministry of Agriculture and Fisheries' (MAF) Glenariffe Hatchery. Fish were collected for seawater transfer experiments during the spring and summer months of two consecutive years (season 2, 1990-1991 and season 3, 1991-1992). The experimental fish were derived from crosses sea-run adults, returning to the spawn. Fertilised ova, alevins, fry, and fingerlings were reared under natural photoperiod and with normal hatchery practices. Initial rearing was in an indoor facility (bore water at 7 °C). When approximately one gram, the young salmon were transplanted to standard, outdoor raceways (30 m long × 3.3 m wide × 1.5 m deep). Glenariffe Stream fed the raceways, with water temperature fluctuating between a minimum of 7 °C (winter) to a maximum of 11-13 °C (summer). On arrival at the Department, the fish were placed into holding tanks and allowed to recover from transportation stresses for a period of 60-70 hours. All tanks were supplied with 'fresh' water flowing at rates that effected complete water exchanges every hour or less. Additionally, water in every experimental tank was gently aerated by compressed air bubbling through air stones. All tanks were covered with plastic netting to prevent fish escape. Fish were not fed during the recovery period to allow complete emptying of the gut.

During season 2, the fish used in the last three seawater challenge tests were marked with an Alcian Blue dye solution. During season 3, all the fish were individually cold-branded. All fish were lightly anaesthetised prior to measurement and marking, to reduce handling stresses, and to ensure good, clean, visible brands were applied. An assumption was made that neither method of marking would affect survival of the fish in seawater.

Effect of improved trophic opportunity on growth

The warmer water of the Departmental Aquarium, and the lower stocking density within the experimental glass tanks, were assumed to improve the prospect of growth for the fish transferred to, and reared in the Department. Therefore, the growth rates of the groups of 'untrained' fish (*see* CHAPTER 3) that were reared for 13 day periods in the laboratory, were compared to growth rates of sibling fish at Glenariffe over the same time periods.

Additionally, a group of 40 'zip-up' fry (taken from Glenariffe on 28 June 1991 - first 'shipment' of season 3) were dip netted to an 80 litre glass tank and reared for up to seven months in 'warm' water (13-15 °C), and at a lower stocking density than sibling fish reared in the colder, spring fed water of Glenariffe hatchery (7-11 °C). These fish were termed the 'accelerated growth' fish. Comparisons were drawn between the growth rate of fish reared at Glenariffe and the accelerated growth fish reared in the laboratory.

Although all the accelerated growth fish were periodically remeasured to assess their individual growth and growth rates, only growth rate data of six individually branded, accelerated growth fish were used in all the comparisons of growth in the Glenariffe reared fish (during the period when fish were collected from Glenariffe for growth experiments, *i.e.* 28 June 1991 - 6 December 1991). These six accelerated growth fish were chosen, at random, from the entire pool of such fish, at the termination of the Glenariffe *versus* laboratory growth experiments (*i.e.* after the 17 December 1991 remeasurement of accelerated fish). The accelerated growth fish were reared in the laboratory until the end of February 1992 (*i.e.* for seven months), whereupon they were killed by overanaesthesia and dissected for morphological and physiological analysis.

Seawater transfer

Initial assessments of seawater tolerance were carried out on all the fish transferred to seawater. A full description of this test is given in the General Methods (CHAPTER 2). Fish were measured for fork length and wet weight. During season 3, standard length and length of the caudal peduncle were also measured. Once measured, the fish were allowed to recover, in aerated freshwater, for at least 30 minutes prior to seawater transfer. All transfers were performed between 10 a.m. and 12 noon. The fish were transferred directly into full strength seawater ($\approx$30‰).

The seawater system used was a closed, recirculated system. Whilst in the Department, the salmon were reared under a 12L:12D photoperiod. The seawater temperature varied slightly (13-16 °C) during the experimental months, as did the salinity (28-38‰). Salinity was far more

stable on a day to day basis. In season 2, fish were transferred to opaque, blue plastic tanks, whereas in season 3, fish were transferred to clear glass tanks.

Fish were observed immediately after transfer, and thereafter every 30-60 minutes over the next 48 hours. General fish behaviour was noted at each observation. Fish that had lost their balance equilibrium, or were lying on the bottom of the tank were removed, and the time of death noted. These fish were killed, if necessary, using a concentrated anaesthetic and dissected. All surviving fish were remeasured at 48 hours post-transfer.

During season 3, six fish from the pool of fish to be transferred to seawater were overanaesthetised to record resting freshwater levels of various physiological parameters ('initial group'). Similarly, at the 48 hour remeasurement, a second group of six fish were also killed for analysis ('48 hour group'), and provided an indication of the physiological status of the fish after 48 hours in seawater. Growth and growth rates were monitored in the remaining fish. These seawater growth fish were termed 'growers'. Growth and growth rates of seawater resident fish were compared with the growth of sibling 'accelerated growth' fish reared in freshwater over the same period.

Effect of salinity on growth

All fish in the growth experiments (fresh- and seawater reared fish) were fed by hand at least five times daily until the end of the growth experiments. Long term growth studies necessitated required that these fish were repeatedly remeasured. On such occasions, the fish were re-branded to ensure individual recognition. Some fish died during the periodic remeasurements from overanaesthesia. Seawater resident and freshwater reared fish were reared in the laboratory until the end of February 1992, whereupon they were killed by overanaesthesia and dissected for analysis.

Fish sampling and dissection

All fish were lightly anaesthetised prior to the measurement of fork length and wet weight (standard and caudal peduncle lengths were also measured during season 3). At the conclusion of the growth experiments, fish were killed by overanaesthesia. Once measured, fish were placed on a cold glass plate and the caudal peduncle severed from the body immediately posterior to the anal fin. Blood was collected from the caudal vasculature into ammonium heparinised micro capillaries. The capillaries were spun at 5000 *g* for six minutes, and blood haematocrit measured. The plasma was transferred to plastic vials and snap frozen in liquid nitrogen. A sample of white muscle was dissected from the tail stump and weighed. The viscera and heart were similarly dissected from the carcass and weighed. These samples were then dried to constant weight. The gills were removed, rinsed in homogenising buffer (Johnson *et al.*, 1977), and snap frozen in liquid nitrogen within 1.5 ml vials. All samples were stored at −80 °C, until further analysis.

Physiological analysis

Blood plasma was analysed for sodium and chloride ion concentration ($mmol.l^{-1}$) and osmolality ($mOsm.kg^{-1}$). Sodium concentration was analysed in a Varian Techtron 1200 Absorption Spectrophotometer. Chloride concentration was determined using a Radiometer CMT 10 chloride titrator. A Wescor Inc. 5100C vapour pressure osmometer was used to determine plasma osmolality. Plasma was pooled, as necessary, prior to analysis, to facilitate that all three determinations were measured for each experimental group. Activity of the enzyme Na^+-K^+-ATPase was measured in the gill samples, using previously described methods (Johnson *et al.*, 1977; Langdon *et al.*, 1984; Franklin, 1989).

Calculation of growth and morphological indices

Fulton's condition factor, $100 \times W/L^3$ (where W is the gram weight, and L is fork length in centimetres), was calculated for each fish at every measurement. Growth rates were calculated for each fish as linear growth rate (LGR), $[L_f - L_i]/t$ (where L_f and L_i are the final and initial fork lengths (mm) respectively, and t is the duration of the growth period in days), and specific growth rate (SGR), $[[\ln(W_f) - \ln(W_i)]/t] \times 100$ (where $\ln(W_f)$ and $\ln(W_i)$ are the natural logarithms of the final and initial wet weights (g) respectively, and t is the duration of the growth period in days). As fish were taken from a single population of fish (raceway) at Glenariffe hatchery, the mean size data (length and weight) of the fish from consecutive 'shipments' were used to calculate growth rates. Specific and linear growth rates were calculated (using the equations above) by subtracting the mean weight or length of the fish from the previous shipment from each fish in the subsequent shipment. Indices of standard length and length of the caudal peduncle were calculated as a proportion of fork length. Cardiac and visceral indices were calculated as a proportion of total weight. Percentage water content of the white muscle and viscera were also calculated. All data were entered into spreadsheet software for calculations and statistical analysis.

Statistical analysis

Results are presented as individual measurements, as the mean $\pm$standard error, or $\pm 95\%$ confidence limits. Statistical significance was assessed using one way analysis of variance (one way ANOVA) and the Standard Error test where applicable. Linear regression was carried out on the growth rate data of laboratory reared freshwater and seawater resident fish. Comparison of the slopes (*b*: the gradient, or regression coefficient) of the regression lines was carried out using a *t*-test (Sokal and Rohlf, 1981). The *t*-test compared the slope and its standard error of the freshwater fish with the slope and standard error of the seawater resident fish. Statistical significance was recorded when $p \leq 0.05$. Some of the data are presented in a graphical form with 'day of the year' represented along the abscissa. The numbers on such axes represent the first day of the month in a 365 day year (*see* DATA HANDLING section and Figure 2.7 in CHAPTER 2).

RESULTS

Unplanned fish death

Eleven seawater challenge tests were carried out in season 2, with six trials providing fish that survived beyond the 48 hour period of the seawater challenge test (25 fish). Six of these fish inexplicably died during the feeding period (one week). Growth rate data from the remaining 19 fish (solid triangles) have been presented in Figures 6.3 and 6.4. However they have not been included in the regression equations and statistical analyses as I feel that the quality of the seawater in the system during season 2 was unsuitable for meaningful study. The seawater system was overhauled and reconditioned between season 2 and 3 and seawater conditions were vastly improved. During season 3, six of the eleven seawater transfer tests provided hatchery reared fish that adapted to seawater (63 fish). Although four of these fish died within the first 48 hours after the period of the seawater challenge test, all the other fish grew well and were reared until 29 February 1992.

Problems arose during the long term growth studies of fish growth experiments (accelerated growth fish and seawater resident fish) due to fish death. Of the 40 accelerated growth fish, exactly half (20 fish) survived for the entire duration of the experiment (28 June 1991 to 24-26 February 1992; seven months). Five fish escaped from the rearing tank, seven fish were unintentionally killed by overanaesthesia and stress of remeasurement, and eight other fish were intentionally killed by overanaesthesia as they were developing 'popeye' (swelling of either eye (or both) due to excessive fluid build up, with occasional internal haemorrhaging within the eye). Popeye is a disease that is relatively common among freshwater reared salmonids in New Zealand and has many causes (Mr NC Boustead, personal communication, 1993). Only the growth rate data (of fish that developed 'popeye') prior to the development of the diseased condition were used in the analyses (*i.e.* growth rate data for the period between the penultimate and ultimate measurements of 'popeye' fish were excluded). Fish that died accidentally during remeasurements were assumed to have grown as well as their (surviving) siblings and were therefore included in the analyses.

Of the 63 fish that survived the six separate transfers to seawater, only 33 survived for the duration of the growth experiments (terminated 26-29 February 1992). Ten fish (transferred to seawater at the same time, *i.e.* during the same seawater challenge trial) were used (20 December 1991) to compare the effects of critical swimming speed (U_{crit}) on blood physiology and gill Na^+-K^+-ATPase activity (*see* CHAPTER 4, Table 4.2). Six fish (transferred to seawater in the same trial) were killed on 3 February 1992 and were used to compare differences between immature and a precociously maturing seawater resident fish (*see* CHAPTER 7). Four fish died within 96 hours of transfer, seven fish died during remeasurements, and three fish escaped from the tanks. As a consequence of these unplanned deaths, a number of assumptions had to be made during the statistical analysis of the data.

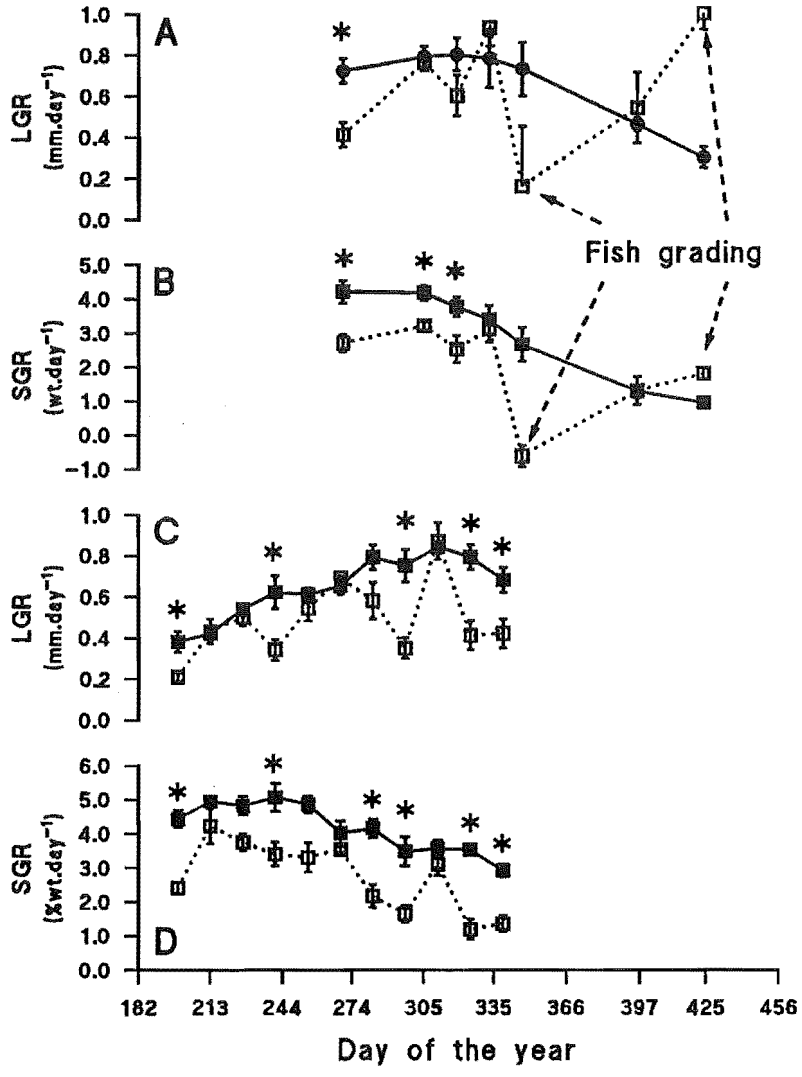


Figure 6.1 Comparison of linear (LGR) and specific (SGR) growth rates of underyearling chinook salmon reared at Glenariffe hatchery (open symbols, dotted lines), and in the laboratory (solid symbols, solid lines). Graphs A and B present data from season 2 (1990-1991), and graphs C and D give data from season 3 (1991-1992). Data are presented as the sample mean ±standard error ($n=6$). Asterisks (*) indicate a significant difference ($p<0.05$, one way ANOVA) between the experimental groups (*i.e.* Glenariffe *versus* laboratory reared fish) on each sampling occasion.

Effect of improved trophic opportunity on growth

During the fortnightly growth experiments of season 2 and 3, the juvenile chinook salmon generally grew faster in the laboratory than at Glenariffe hatchery (Figure 6.1). Data in Figure 6.1 compare growth rates (linear, LGR and specific, SGR) realised by the fish during the growth rate experiments of season 2 and 3. Data for the second growth trial of season 2 have been omitted as the laboratory reared fish were fed the wrong feed size, and exhibited very poor growth (*see* CHAPTER 3, Figure 3.3). In season 2, the raceway population was graded for equality of size on two occasions as indicated in graphs A and B. As the size structure of the raceway population would have been altered as a result, it was not possible to statistically assess

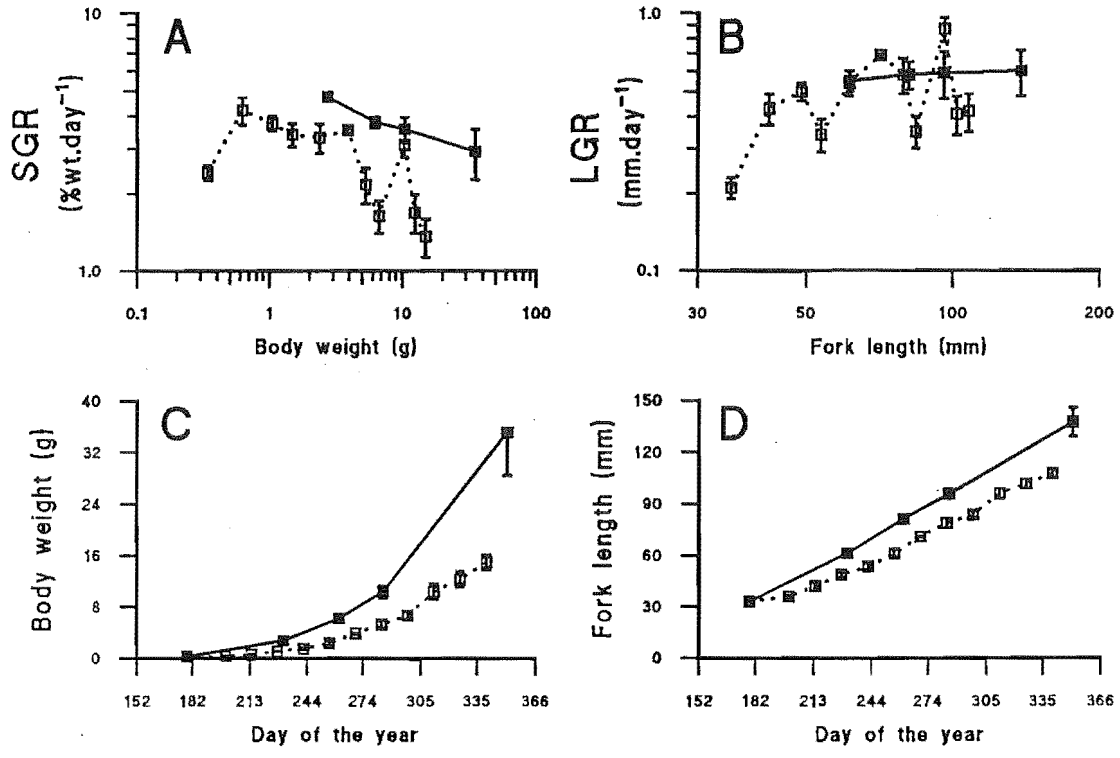


Figure 6.2 Comparison of specific growth rate (SGR, graph A) and linear growth rate (LGR, graph B) against fish size (body weight and fork length respectively) for accelerated laboratory (solid symbols, solid lines) and Glenariffe hatchery (open symbols, dotted lines) reared fish. Both axes in graphs A and B have logarithmic scales. Graphs C and D compare actual size (body weight, graph C; fork length, graph D) attained by the accelerated growth and Glenariffe hatchery fish against sampling date (day of the year). Data are from experiments carried out during season 3.

the data in the last three growth trials of season 2. The raceway population used in season 3 was not graded during the period of investigation.

One way analyses of variance were performed on the growth rate data of the two groups (hatchery *versus* laboratory reared fish) for each season (first four pairs of data points of season 2 (A and B), and all trials of season 3 (C and D), Figure 6.1). Although there was no significant difference ($p=0.17$) in LGR between the groups during season 2, SGR was significantly greater ($p<0.001$) in the laboratory reared fish. During season 3, both LGR and SGR were significantly elevated ($p<0.0001$, for both parameters) in the laboratory reared fish. It is evident that though the growth rates of fish reared at Glenariffe were occasionally equivalent to the laboratory fish, they were never higher.

Growth rate of underyearling chinook salmon was greatly accelerated in the fish that were reared in the laboratory on a long term basis, as shown in Figure 6.2. The larger accumulated mass and length of laboratory fish was a result of a steady and consistent daily growth rate that was often higher, sometimes equal but never lower than the less stable daily growth rates of the Glenariffe fish. Overall, linear and specific growth rates were 0.55 mm.day^{-1} and 2.76 %body

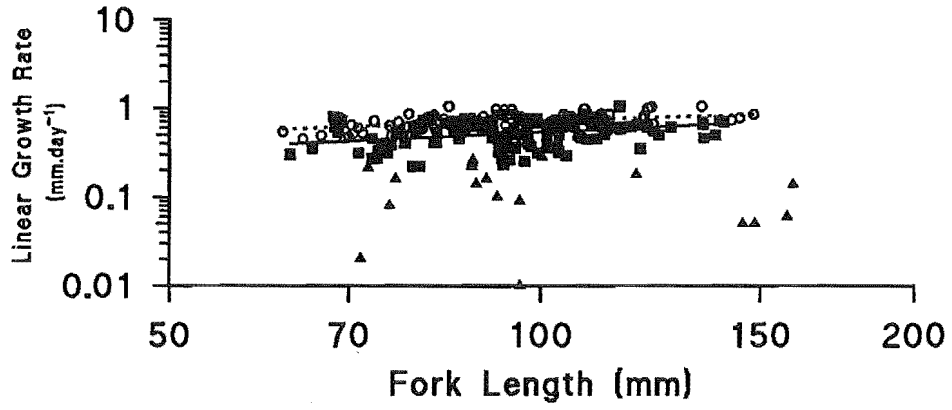


Figure 6.3 Individual linear growth rates (LGR, mm.day^{-1}) of laboratory reared freshwater (open symbols, dotted line) and seawater (solid squares, solid line, season 3; solid triangles, season 2) chinook salmon against fork length (mm). Both axes have logarithmic scales. Data for seawater resident fish from season 2 have not been included in the seawater growth regression analysis.

weight.day^{-1} for the accelerated fish (28 June to 17 December 1991), and 0.46 mm.day^{-1} and 2.57 %body weight.day^{-1} for Glenariffe reared fish (28 June to 6 December 1991). At the conclusion of the examination period the accelerated fish were approximately 135% heavier (35.17±6.70 g (mean±95%CL) *versus* 14.94±1.17 g), and 28% longer (137.54±8.31 mm *versus* 107.79±2.5 mm) than the Glenariffe fish.

Effect of salinity on growth

In order to compare growth rates of laboratory reared seawater resident fish with freshwater reared fish, growth rate data for both groups were compiled and are presented in Figure 6.3 (LGR) and Figure 6.4 (SGR) against fish size. The freshwater growth rate data came from similar sized fish that were reared in the laboratory (*i.e.* the 'untrained' fish of Chapter 3 - data from season 3 only, and accelerated growth fish data). Growth rates of laboratory reared fish were used (as opposed to data for Glenariffe reared fish) as the temperature of freshwater in the laboratory (13-15 °C) was similar to that of the seawater system (13-16 °C) over the period of investigation.

Although both freshwater and seawater resident fish were reared until the end of February 1992, only growth rates that were calculated from fish measurements prior to the end of December 1991 were used in the comparison. The fish were fed periodically during the 'holiday period' (by myself, other students, and university security staff), however, they were not fed as frequently as they were outwith that period. Because the data collected during that period were from fish that had experienced a different 'treatment', they were omitted from the analysis. Growth rates decreased over the Christmas period, and did increase thereafter, once 'normal' feeding rates were resumed.

Figure 6.3 presents the linear growth rate data for the freshwater and seawater resident fish. Linear growth rate increased with fork length over the size range (*see* CHAPTER 3,

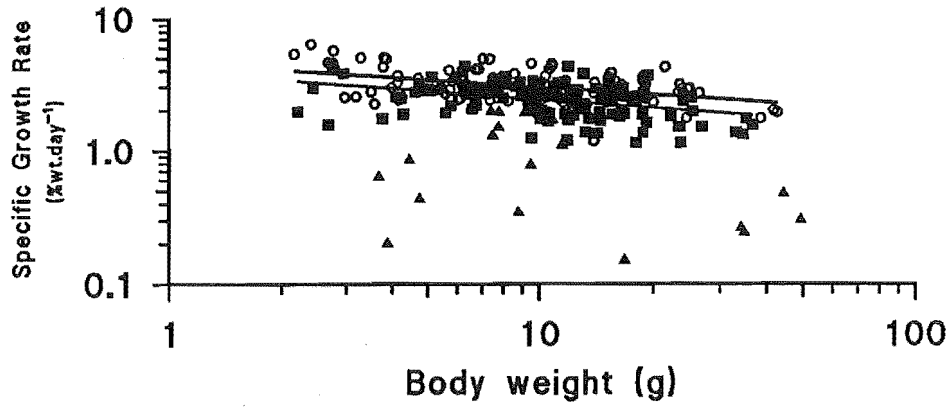


Figure 6.4 Individual specific growth rates (SGR, %body weight.day^{-1}) of laboratory reared freshwater (open symbols, dotted line) and seawater (solid squares, solid line, season 3; solid triangles, season 2) chinook salmon against body weight (g). Both axes have logarithmic scales. Data for seawater resident fish from season 2 have not been included in the regression analysis.

Figure 3.3). The relationship between LGR and fork length was significant for both groups ($p=1.07\times10^{-4}$ for FW, and $p=8.07\times10^{-5}$ for SW), and is given by the equations $\log Y=0.438(\log X)-1.02$ ($r^2=0.170$, $n=92$) for the freshwater resident fish and by $\log Y=0.655(\log X)-1.58$ ($r^2=0.115$, $n=125$) for the seawater resident fish. Statistical comparison of the slopes of the regression lines indicated that there was no difference ($p>0.05$) in growth rate between the two groups (t-test).

The specific growth rates of freshwater and seawater resident fish decreased with fish size, over the range where comparisons were possible (Figure 6.4). The relationship between SGR and body weight was significant for both groups ($p=4.11\times10^{-6}$ for FW, and $p=3.67\times10^{-6}$ for SW), and is given by the regression line $\log Y=-0.191(\log X)+0.664$ ($r^2=0.211$, $n=92$) for freshwater fish and by $\log Y=-0.215(\log X)+0.594$ ($r^2=0.161$, $n=125$) for seawater fish. A statistical comparison of the slopes of the two regression lines indicated that there was no difference ($p>0.05$) in growth rate between the groups (t-test).

It was found that the indices of standard length and the caudal peduncle length changed with fish growth in freshwater reared fish (*see* CHAPTER 3, Figure 3.4). Comparisons of both indices between freshwater and seawater resident fish were made by restricting the seawater data to that collected on the last measurement of those fish (26-29 February 1992, $n=33$). In this way, all the seawater reared fish had been resident in seawater for at least two months, and therefore any (possible) changes would have had time to develop. Data for the indices in freshwater fish ($n=50$) were collected from all the measurements of fish within the same size (fork length) range (range of seawater resident fish at death was 116.90-157.5 mm). Furthermore, all the index of standard length, and index of caudal peduncle length data, have been arranged into fork length classes of 10 mm (*i.e.* 110-119.99 mm, *etc.*).

One way analyses of variance between the fork length data of freshwater and seawater resident fish, within each size class, indicated that the size data were equivalent ($p>0.05$) within

all the classes. Therefore it was possible to directly compare the indices between each freshwater and seawater resident group. No statistical differences in any of the size classes were evident ($p > 0.05$, one way ANOVA) between every pairing of freshwater and seawater resident fish, in each size group, Table 6.2. The data in the 160-169.99 mm size class were analysed using the Standard Error test.

Table 6.2 Comparison of the indices of length of the caudal peduncle (CP I) and standard length (StdL I) between freshwater and seawater resident fish, within restricted fork length ranges. Data are presented as the sample mean ±95% confidence limits (n is variable, and is given within parentheses for each group, beneath the fork length data).

	FRESHWATER REARED FISH			SEAWATER REARED FISH		
Fork Length Size Range	Fork Length (mm)	CP I (%FkL)	StdL I (%FkL)	Fork Length (mm)	CP I (%FkL)	StdL I (%FkL)
110 to 119.99 mm	116.30±4.37 (5)	13.18±0.42	90.49±0.81	116.09±7.62 (3)	12.73±0.35	89.93±1.22
120 to 129.99 mm	124.54±2.71 (16)	12.58±0.35	91.44±0.34	127.49±2.87 (12)	12.77±0.99	90.39±0.36
130 to 139.99 mm	136.50±2.66 (9)	12.39±0.37	90.20±0.53	135.85±2.60 (7)	12.91±0.31	90.62±0.12
140 to 149.99 mm	144.87±2.21 (10)	12.88±0.66	90.62±0.20	145.53±3.02 (6)	12.29±0.27	90.37±0.22
150 to 159.99 mm	153.55±3.37 (3)	12.14±0.56	90.14±1.31	156.78±1.78 (4)	12.20±0.83	89.85±0.67
160 to 169.99 mm	165.02±3.05 (7)	12.75±0.68	90.45±0.35	161.50 (1)	13.09	90.77

The seawater growth experiments were ended on 26-29 February 1992, and 33 seawater resident fish were dissected. The post-mortem morphological data (visceral and heart weight, visceral and cardiac indices, and percentage water content of the viscera and the white muscle) were compared to data from freshwater reared fish of a similar size (weight) range (range of seawater resident fish at death was 20.16-54.29 g). Data for 33 freshwater fish were randomly picked from the entire data set of similarly sized freshwater fish (accelerated growth, and fortnightly growth trial groups of season 2 and 3). The fork length and body weight data were compared with analyses of variance and were not different ($p > 0.05$ in both tests, one way ANOVA). Data comparing the viscera weight (A) and heart weight (B) of seawater resident and freshwater reared fish are presented in Figure 6.5, against body weight.

Viscera (Figure 6.5A) and heart weight (Figure 6.5B) scaled with total body weight. Viscera weight is given by the regression line $\log Y = 0.698(\log X) - 0.462$ ($r^2 = 0.678$, $n = 33$) for seawater resident fish, and by $\log Y = 0.696(\log X) - 0.541$ ($r^2 = 0.752$, $n = 33$). Heart weight is

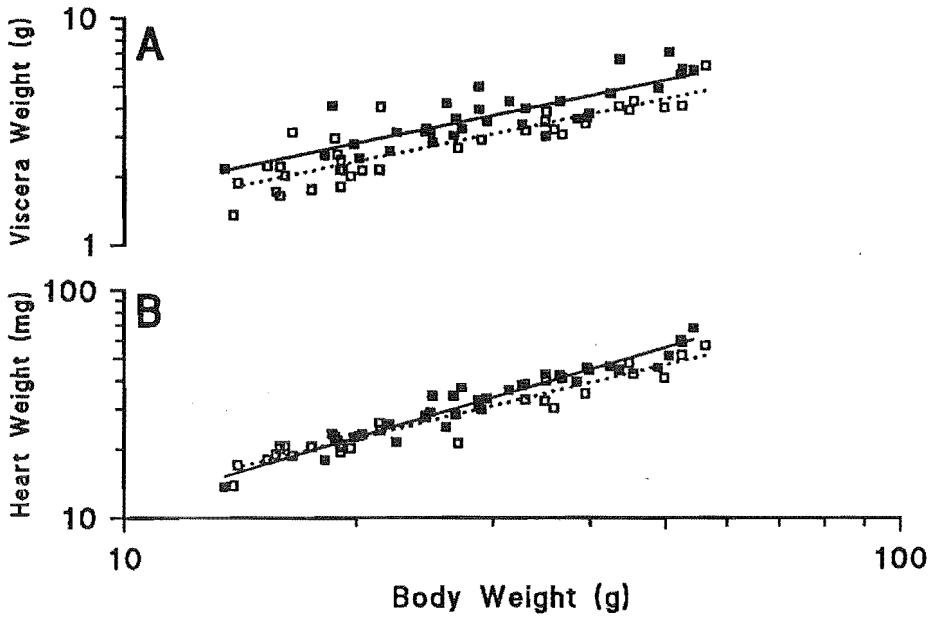


Figure 6.5 Plots of viscera (A) and heart (B) weight against body weight for seawater resident (solid symbols, solid lines) and freshwater reared (open symbols, dotted lines) underyearling chinook salmon. Both axes have logarithmic scales. Data points of seawater resident fish represent measurements of individual fish from season 3. Data points for freshwater reared fish are from individual fish from both season 2 and 3.

given by the regression line $\log Y = 0.989(\log X) - 0.065$ ($r^2 = 0.931$, $n = 33$) for seawater resident fish, and by $\log Y = 0.809(\log X) - 0.294$ ($r^2 = 0.930$, $n = 33$). No significant differences ($p > 0.05$) were found between the slopes of the viscera and heart weight regression lines of freshwater and seawater reared fish data (*t*-test).

Viscera weight of seawater resident fish (3.92 ± 0.43 g, mean$\pm$95%CL, $n = 33$) was significantly greater ($p < 0.001$, one way ANOVA) than in freshwater fish (2.86 ± 0.37 g). There was no difference between the heart weight data ($p > 0.05$, one way ANOVA, 33.92 ± 4.40 mg, SW; 30.49 ± 4.96 mg, FW). The seawater fish had a visceral index of $12.58 \pm 0.95\%$, statistically greater ($p = 0.03$, one way ANOVA) than the index of freshwater fish, which was $11.13 \pm 0.97\%$. No difference ($p = 0.21$) was found between the cardiac indices of the two groups ($0.112 \pm 0.002\%$ for SW fish, and $0.109 \pm 0.005\%$ for FW fish).

Percentage water content of the viscera differed between the groups as shown in Figure 6.6A. Over the range, average water content of the viscera of seawater resident fish was $72.21 \pm 1.58\%$, whereas that of the freshwater resident fish was $65.36 \pm 1.41\%$. The difference was highly significant ($p < 0.0001$, one way ANOVA). No difference ($p = 0.09$, one way ANOVA) was found between the percentage water content of the white muscle between the groups ($79.04 \pm 0.16\%$ for SW fish, and $78.77 \pm 0.27\%$ for FW fish; Figure 6.6B).

The physiological variables, gill Na^+-K^+-ATPase, blood haematocrit, plasma osmolality, and plasma concentrations of sodium and chloride were assumed to be independent of body size. Gill Na^+-K^+-ATPase activity in particular was found to vary with season (*see* CHAPTER 3,

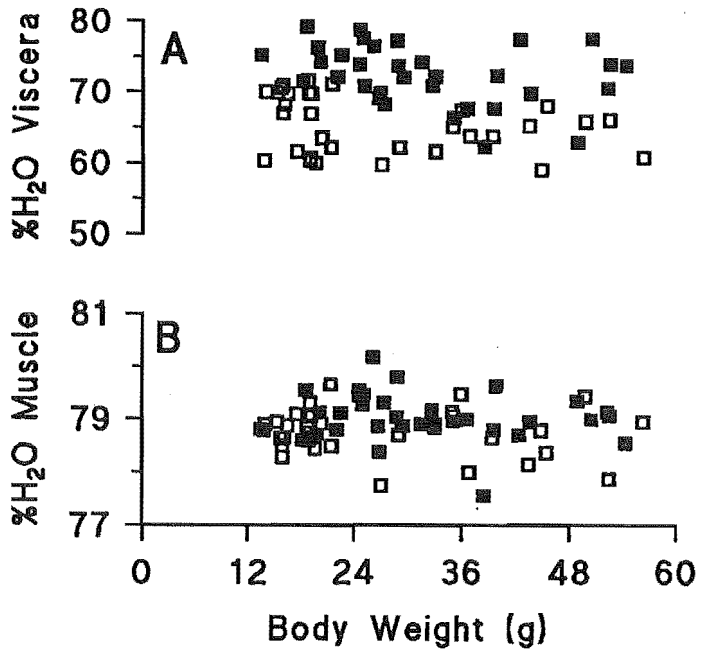


Figure 6.6 Plots of percentage water content of the viscera (%H_2O Viscera, graph A) and white muscle (%H_2O Muscle, graph B) against body weight of underyearling chinook salmon. Data are from season 3, and each point represents measurements from an individual fish. Solid symbols represent seawater resident fish, open symbols freshwater reared fish.

Figures 3.7 to 3.10, and CHAPTER 5, Figures 5.1 and 5.2). For these reasons, statistical comparison of the physiological data obtained from seawater resident and freshwater reared fish was restricted to the measurements made on the fish at the completion of the growth experiments (end of February 1992, n=33 for SW fish, and n=20 for FW fish). Both groups contained sibling fish of the same age (Table 6.3).

The physiological 'make up' of the seawater resident fish was quite different to that of the freshwater reared fish with regard to all the parameters in Table 6.3, excepting haematocrit. The highest levels of gill Na^+-K^+-ATPase activity were recorded in the seawater resident fish, and were far higher than those measured in the freshwater fish ($p<0.0001$, one way ANOVA) killed at the same time. All the seawater resident fish that were analysed had gill Na^+-K^+-ATPase activities greater than 10 μmol P_i.mg protein^{-1}.h^{-1} (range 10.38 – 16.49 μmol P_i.mg protein^{-1}.h^{-1}). Furthermore, gill Na^+-K^+-ATPase activity of seawater resident fish was almost double that of their sibling fish in freshwater at the same time (Table 6.3).

Resting blood haematocrits of seawater resident and freshwater reared fish were not significantly different (p=0.17, one way ANOVA, Table 6.3). A similar result was found between 'initial' and '48 hour' fish during the period when the chinook survived the seawater challenge tests (*see* CHAPTER 5, Figure 5.5). Plasma osmolality, and plasma concentrations of sodium and chloride were significantly elevated in the seawater resident fish ($p<0.001$, $p<0.01$, and $p<0.0001$ respectively, one way ANOVA, Table 6.3).

Table 6.3 Physiological data (gill Na^+-K^+-ATPase activity [μmol P_i.mg protein^{-1}.h^{-1}], blood haematocrit [percent red cell volume], plasma osmolality [mOsm.kg^{-1}], and plasma sodium and chloride concentration [mmol.l^{-1}]) for the groups of seawater resident, and freshwater reared, underyearling chinook salmon. Data are presented as the sample mean ±95% confidence limits (n=33 for the seawater resident fish, and n=20 for the freshwater reared fish). Statistical significance between the groups is given by asterisks (*) when $p<0.01$, by daggers (†) when $p<0.001$, and by double daggers (‡) when $p<0.0001$.

Physiological parameter	SEAWATER RESIDENT CHINOOK	FRESHWATER REARED CHINOOK
Na^+-K^+-ATPase	13.04±0.63 ‡	7.23±0.76
Haematocrit	39.07±1.00	37.66±2.18
Osmolality	335±6 †	317±8
Sodium	164±4 *	154±3
Chloride	130±3 ‡	123±2

DISCUSSION

Effect of improved trophic opportunity on growth

The rearing environment of the laboratory generally accelerated the growth rate of the underyearling chinook salmon used in this study (Figures 6.1 and 6.2), most significantly through increases of weight specific growth rate (SGR). The effect was clearly evident in the group of 'accelerated' fish that were reared in the laboratory from the 'zip-up' stage (Figure 6.2). These fish were considerably larger at the end of the experimental period (172 days after transfer to the laboratory) and had attained more than double the weight, and 30 mm more length, than their Glenariffe siblings. Growth rates of poikilothermic animals generally increase with 'mild' increases in ambient temperature (Brett, 1979; Brett and Groves, 1979). It is not possible to say whether temperature alone caused the elevated growth rates, as laboratory fish were also reared under an artificial photoperiod, and at different stocking densities and feeding regimes. Regardless of the cause, the water temperature and feeding regime that was afforded the fish reared in the laboratory, particularly those fish that were reared long-term, resulted in enhanced growth rates above those attained by Glenariffe hatchery reared fish. With recourse to the literature I would suggest that temperature and improved individual feeding ability would have enhanced growth to a greater extent than a reduced stocking density.

The temperature of the laboratory freshwater system was 13-14 °C during the period of investigation. Brett (1979) gave evidence that the temperature range within which juvenile salmonids exhibit fastest growth was 10-20 °C, depending on the species. Given a natural choice (Power and Shooner, 1966), or under laboratory conditions (Peterson and Metcalfe, 1979; Peterson *et al.*, 1979), juvenile Atlantic salmon, *Salmo salar*, preferred water temperatures of

15-17 °C. Banks and associates (1971) reported maximum freshwater growth rates of chinook salmon, *Oncorhynchus tshawytscha*, occurred when the temperature was 15.5 °C. Zaugg and associates (1985) state in their discussion that the growth rate of fall chinook salmon is extremely rapid when the water temperature is between 11 °C and 15 °C.

Growth rates of coho salmon, *Oncorhynchus kisutch*, have been accelerated using 'warm' water for stocking programmes and sea-cage rearing in Puget Sound, Washington, D.C. since 1967 (Donaldson and Brannon, 1975). Brannon and co-workers (1982) reported that coho salmon could be successfully released as 'zero-age' smolts (coho smolts are normally yearlings) following accelerated freshwater rearing. They also reported that the accelerated smolt tended to return to spawn at younger ages (2 year old fish). Research by Saxton and associates (1983) reported later work on coho indicating that predictions of saltwater performance were not possible using criteria that assessed 'smolt condition' of heat accelerated fish. Atlantic salmon have been reared in warm, hydroelectric waste water in Russia (Kazakov *et al.*, 1988) and geothermal water in Iceland (Ísaksson, 1985). The age at which the fish became smolts was reduced in both these studies, and have yielded significant increases to the respective fisheries. Other studies have indicated that the time of parr-smolt transformation may be advanced following accelerated rearing in freshwater (Zaugg *et al.*, 1972; Zaugg and McLain, 1976; Clarke *et al.*, 1978; Ewing *et al.*, 1979; Ewing, Pribble *et al.*, 1980; Johnston and Saunders, 1981; Virtanen *et al.*, 1981).

Increased densities (biomass of fish per unit area or volume) of salmon during hatchery rearing can reduce the production and performance of coho salmon (Schreck, 1982b; Schreck *et al.*, 1985), Atlantic salmon (Reftsie and Kittelsen, 1976), rainbow trout, *Oncorhynchus mykiss* (Reftsie, 1977; Holm *et al.*, 1990), brown trout, *Salmo trutta* (Brown, 1946a,b), brook charr, *Salvelinus fontinalis* (Vijayan and Leatherland, 1988), and Arctic charr, *Salvelinus alpinus* (Jobling, 1985). However, other research has demonstrated that rearing density had no effect on growth or performance of coho salmon (Fagerlund *et al.*, 1987), Atlantic salmon (Soderberg and Meade, 1987; Kjartansson *et al.*, 1988), or chinook salmon (Carl, 1984).

The data for Glenariffe reared fish of season 3 presented in Figure 6.1 give the impression that the growth rates of underyearling chinook salmon fluctuated in a cyclical manner. Farbridge and Leatherland (1987a,b,c) reported that the growth rates of rainbow trout and coho salmon described cyclical periods of rapid and slow growth that were in tune with the lunar cycle. They also showed that growth in length, and growth in mass were possibly 'out of phase' with each other. In their papers, the periodicity between growth rate peaks was around 14-15 days. Similar cyclical rhythmicity regarding fish growth rates were recorded by Brown (1946b) and Swift (1961) for brown trout, and Wagner (1974) for rainbow trout. In Figure 6.1 (graphs C and D), the points are separated by fortnightly periods, and therefore the periodicity between the apparent peaks is around 42 days. The drop in linear growth rate (LGR) at the fourth sampling occasion (Figure 6.1, graph C) corresponds to the time when the chinook were transferred from the indoor hatchery trough to the outdoor raceway. Once in the raceway, the fish were fed at a constant rate during the remainder of the examination period.

Furthermore, gill disease outbreaks were not reported during this time (Mr JRE Sykes, personal communication, 1993). Therefore, although the cyclical patterns of growth are apparent in the data, they may possibly be due to seasonal artifacts (*e.g.* freshets and cloudy water). Nevertheless, the possibility of rhythmicity regarding growth rate would be interesting to follow up with further study.

Effect of salinity on growth

Only growth rate data of the fish transferred to seawater during season 3 have been used in the statistical comparison of growth rate *versus* salinity. As discussed in Chapter 5, the disparity between the data from season 2 and 3 may be explained by the quality of the seawater in the closed, recirculated, seawater system in the Department.

The ability of underyearling chinook salmon 'smolts' to grow in seawater, relative to sibling fish growing in freshwater was investigated over a period of five months (October 1991 to February 1992). Linear and specific growth rates of seawater resident and freshwater reared fish did not differ with respect to salinity (Figures 6.3 and 6.4). These results are in accordance with previous studies. Early work and observation indicated that transfer to the marine environment was essential for survival (Koch *et al.*, 1959) and continued growth (Hoar, 1939; Power, 1959) of young salmon. Eriksson and Lundqvist (1982) reported that Baltic salmon (*Salmo salar*) fed surplus rations, and kept under conditions of constant photoperiod (12L:12D) and temperature (11.0±0.5 °C) exhibited periodic fluctuations of high and low condition factor, which were associated with changes of parr- and smolt-like appearances. Their study indicated that an endogenous rhythm exists within salmonids with respect to development of hypo-osmoregulatory ability and seawater survival. However for parr-smolt transformation to proceed 'normally' at definite, predictable periods, it must be modulated by the environmental '*zeitgebers*' (cues, prompts). Furthermore, it is now recognised that salmon (all species) do not require a transfer to seawater for their continued growth and survival (Berg, 1979; Collins, 1975; Peden and Edwards, 1976; Martin *et al.*, 1981).

Many studies have reported the incidence of 'smolt-parr reversion', or 'desmoltification' in young salmonid smolts prevented from reaching the ocean by some means or other. The ability to survive a transfer to seawater is transient, and therefore 'the smolt' represents only a brief (recurring) period of development. It would appear that the 'environmental salinity' regulates whether the factors associated with the 'smolt condition' (seawater tolerance) are maintained, or whether the physiology of the fish returns to a state more consistent with freshwater and the 'parr condition' (Conte and Wagner, 1965; Zaugg and McLain, 1970, 1972; Mahnken, 1973; Zaugg and Wagner, 1973; Lasserre *et al.*, 1978; Epstein *et al.*, 1980; Boeuf and Harache, 1982; Gorbman *et al.*, 1982; Koch, 1982; Langdon, 1985; Beckman and Zaugg, 1990).

Growth of seawater adapted salmonids, in varying environmental salinities, relative to growth of siblings in freshwater has been investigated previously. Not one of the studies has reported enhanced growth of fish in full strength seawater (reviewed by Brett, 1979). The

reported data however, are conflicting, and in many cases fish appear to have been used that had not undergone parr-smolt transformation, and therefore by definition were probably unable to withstand the marine environment. Salmonid growth rates have most commonly been compared between fish reared in freshwater ($\approx$20 mOsm.kg^{-1}) isotonic seawater ($\approx$8-10‰ seawater, $\approx$300 mOsm.kg^{-1}) and/or full strength seawater ($\approx$30‰ seawater, $\approx$1000 mOsm.kg^{-1}). The coho salmon is possibly the most frequently studied salmonid in this regard.

Canagaratnam (1959) and Otto (1971) both reported that faster growth of underyearling coho was achieved in isotonic salinities (12‰, and 10% seawater respectively) compared to freshwater. Clarke and associates (1981) found no difference in the growth rate of underyearling coho, chinook or sockeye salmon fry in salinities up to 20‰, but growth rates of chinook and sockeye were retarded at a higher salinity (29‰). Bullivant (1958, 1961) and Kepshire and McNeil (1972) found that growth rates of chinook were maximal in half-strength (15-18‰) seawater. In contrast, Morgan and Iwama (1991) found that increasing salinities (5-28‰), and particularly those above isotonic salinity, decreased growth rate relative to that achieved in freshwater, for the fry of rainbow and steelhead trout, and chinook salmon. McKay and Gjerde (1985) found similar results for rainbow trout as did Nahhas and co-workers (1982a), although one could question whether the stock of fish they worked on was anadromous, and whether transfer to seawater occurred during the physiologically optimum time (*i.e.* during the period of parr-smolt transformation). Smith and Thorpe (1976) however, reported that the annual growth rate of rainbow trout was higher in seawater than in freshwater, when the fish were fed *ad libitum* rations. Saunders and Henderson (1969) reported growth of Atlantic salmon smolts was equally fast in freshwater and brackish (15‰), but significantly slower in 30‰ seawater. Saunders and associates (Shaw *et al.*, 1975b; McCormick *et al.*, 1989) however, subsequently found reduced growth in Atlantic salmon smolts in isotonic salinities (10‰ seawater) compared to growth in freshwater and full strength (30‰) seawater.

In some of the studies given above, routine metabolic rates were assessed for the fish in the different treatment groups. Metabolic rates have been found to be lowest in freshwater (Morgan and Iwama, 1991), lowest in isotonic salinity (Rao, 1968), or unaffected by salinity (Bullivant, 1961). Power (1959) recorded that basal metabolic rates of Atlantic salmon smolts were lower than those of parr, at all water temperatures below 13.5 °C. In short therefore, the published work concerning the effect of salinity on growth rate and metabolic rate is rather conflicting and confusing. However this is probably due to the logistical difficulties of controlling all the experimental conditions and that each individual investigation would have had slightly different objectives.

Usher and associates (1990, 1991) have demonstrated that appetite, digestibility (of nitrogen), and gut function in Atlantic salmon smolts was reduced for up to a month post transfer. The decrease in food assimilation reduced growth rates considerably over the first 40 days of their experiment. Growth rates thereafter were equivalent between their groups. The chinook used here generally made feeding strikes within 24-48 hours of the seawater challenge test (*i.e.* within 96 hours post transfer). Additionally, the fish fed as voraciously as their

freshwater counterparts, even taking feed that had settled at the bottom of the tanks, something that was never observed with the freshwater reared fish. Quantitative assessments of appetite, feed consumption rates, gut evacuation rates, and feed assimilation were not undertaken. In the light of the differences manifest between chinook salmon and Atlantic salmon with regard to seawater adaptation and feeding, such analyses would be worthwhile.

In their natural environment, chinook salmon make extensive use of estuarine areas prior to migrating to the sea proper (Reimers, 1973; Healey, 1980a,b, 1982, 1983; Kjelson *et al.*, 1982). Although New Zealand rivers do not afford the same stable estuarine environments that are common on North American rivers, chinook in this country also utilise these areas for feeding (Davis *et al.*, 1983; Eldon and Greager, 1983; Eldon and Kelly, 1985; Davis and Unwin, 1989). Whether chinook use these areas primarily for the improved prey availability, or for adaptation to the brackish and marine environment is uncertain. However, rapid growth has been recorded in estuarine environments (Reimers, 1973; Healey, 1980; Kjelson *et al.*, 1982). The results presented here suggest that chinook salmon 'smolts' are able to survive and grow well following a direct transfer to full strength seawater (Franklin, 1989; and *see* CHAPTER 5, Figures 5.1 and 5.2).

Heart and viscera weights scaled with body weight. The viscera of seawater resident fish were much heavier than those of freshwater reared fish. The viscera also accounted for a greater proportion of the body weight (visceral index of seawater resident fish was $12.58 \pm 0.95\%$, whereas that of freshwater reared fish was 11.13 ± 0.97). This may in part be explained by the higher water content in the viscera of the seawater resident fish (72.211.58% water, compared to $65.36 \pm 1.41\%$ water in the freshwater reared fish). Seawater resident fish necessarily drink seawater to obtain water, excreting the 'salt load' via the gills (Na^+-K^+-ATPase 'pumps'), or through production of an isotonic, or slightly hypertonic urine (Holmes and Stainer, 1966; Potts *et al.*, 1970; Eddy and Talbot, 1985; Usher *et al.*, 1988). Therefore the increased water content of the seawater resident fish viscera may reflect this elevated drinking rate. Alternatively, the differences in visceral water content between the groups could be explained by adoption of differing storage routes for surplus energy. The freshwater reared fish had increasingly large fat deposits, noticed at dissection, and in liquid lipid pools whilst the samples were being desiccated at 70 °C. Such 'lipid stores' were not observed in the viscera of seawater resident fish.

Usher and co-associates (1991) have observed differences in the proximate analysis of seawater resident (three months) and freshwater reared Atlantic salmon. Approximate values of percent water content (whole carcass) were 68% for the freshwater fish, and 76% for the seawater resident fish (*see* Figure 13 in Usher *et al.*, 1991). They contest that the metabolism of the seawater resident post-smolts favoured protein deposition, whereas that of the freshwater reared post-smolts favoured lipid deposition. The difference in the route of energy storage would have adaptive value for fish overwintering in freshwater, as food is limited during that time, and appetite depressed due to colder water temperature (Metcalfe *et al.*, 1986; Usher *et al.*, 1991). Smith and Thorpe (1976) have also reported differing metabolic pathways dependent

on salinity, demonstrating that the efficiency of nitrogen retention is higher in seawater adapted, than freshwater adapted rainbow trout.

The physiological measurements of the fish at the end of the growth experiments provide data that are in accordance with earlier work. Increases in gill Na^+-K^+-ATPase activity with seawater adaptation and residence have been reported for both *Salmo* and *Oncorhynchus* on both sides of the Atlantic (Giles and Vanstone, 1976a; Dickhoff *et al.*, 1977, 1989; Folmar and Dickhoff, 1978, 1979; Langdon and Thorpe, 1984, 1985; McCormick *et al.*, 1987; Hoar, 1988; Madsen and Naamansen, 1989). In addition, gill Na^+-K^+-ATPase activities of seawater adapted New Zealand chinook, and sockeye salmon increased with seawater residence in a previous study (Franklin, 1989). The procedure used to measure gill Na^+-K^+-ATPase activity in this study was similar to that employed by Franklin (1989). He also found that enzyme activity of chinook (and sockeye) salmon increased some three to four fold with seawater residence. His fish were reared in seawater for 30 days.

Blood haematocrit was similar in seawater resident and freshwater reared fish. Franklin (1989) found that blood haematocrit did not differ after 30 days of seawater residence from that of sibling fish killed prior to seawater transfer. Blackburn and Clarke (1987) state that blood haematocrit is not a reliable indicator of likely seawater survival after a 24 hour seawater challenge test. It follows therefore that haematocrit in fish that are fully adapted to their environment are unlikely to differ (Morgan and Iwama, 1991). Whilst haematocrit (percent red cell volume of the whole blood) does not differ between seawater resident and freshwater reared fish, electrophoretic analysis of the haemoglobin indicates that during parr-smolt transformation the composition of the haemoglobins within the erythrocytes change markedly, both in *Salmo* (Koch, 1982) and *Oncorhynchus* species (Vanstone *et al.*, 1964; Giles and Vanstone, 1976b; Giles and Randall, 1980; Bradley and Rourke, 1984). The changes are thought to be pre-adaptive, with regard to blood-gas transport, for the transformation of environment and life-history pattern.

Plasma concentration of sodium and chloride ions, and plasma osmolality increased in the seawater resident fish. The plasma values for seawater resident fish obtained in this study (*i.e.* $[Na^+]_{pl} < 180$ mmol.l^{-1}, $[Cl^-]_{pl} < 160$ mmol.l^{-1}, and $[\text{Osmolality}]_{pl} < 360$ mOsm.kg^{-1}, Table 6.3) are in accord with those published elsewhere (Eddy and Bath, 1979; Boeuf and Harache, 1982; Johnston and Cheverie, 1985; Blackburn and Clarke, 1987; Kjartansson *et al.*, 1988; Sandnes *et al.*, 1988; Stagg *et al.*, 1989; Bergheim *et al.*, 1990; Morgan and Iwama, 1991; Usher *et al.*, 1991), and indicate that the fish were regulating blood ions effectively against their concentration gradients.

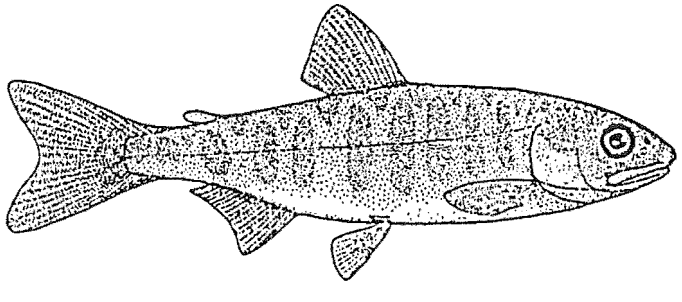
In summary, the results presented here and those published previously do not account for the fact that salmonids reared in seawater attain a larger size than fish held in freshwater. Flain (1972a) reported the size of two year old wild, sea-run chinook salmon as being 58 cm for fork length, and 2.3 kg wet weight (Table 6.1). More recent data from sea-run ocean ranched fish (released as yearling smolts at ≈ 50 g) indicate that the size range of two year old fish is slightly less than reported by Flain (1972a) for wild stocks. The fork length range was given as

48-52 cm, and the body weight range as 1.9-2.5 kg (Mr JRE Sykes, personal communication, 1993). Chinook salmon in New Zealand have become slightly smaller since the advent of hatchery releases (ocean ranching), although the reasons why this has occurred are not known (Unwin, 1991). Two year old fish reared in a freshwater hatchery environment (Peacock Springs Salmon Farm, Ltd) had a mean fork length of 44.8 cm, and weighed 1.4 kg (*see* CHAPTER 3, Figure 3.1). Moreover, two year old fish, fed maximum rations (2% body weight per day) and reared at Glenariffe hatchery were approximately 42 cm (fork length) and 1.2 kg (body weight; Mr CL Hopkins, personal communication, 1993). Both the Glenariffe hatchery and the Peacock Springs chinook (wholly freshwater reared fish) are somewhat smaller than sea-run siblings of the same age.

Smith and Thorpe (1976) argued that the higher growth rates observed in their seawater adapted rainbow trout resulted from those fish sustaining the elevated rate of growth, characteristic of the parr-smolt transformation period beyond the smolt stage, whereas the fish that were retained in freshwater, exhibited progressively slower growth. Brett (1979) reviewed the published research on salmonid growth and salinity and stated that "*such an enhanced ability to grow appears to characterise the marine stage, quite separate from any circumstance of abundance of food.*" Furthermore, Koch (1968) stated that "*then forced to do so, all the individuals of SALMO SALAR are able to live permanently in fresh water, but apparently their growth rate is generally reduced.*" The need for experimentally controlled (temperature), large scale (annular tank) experiments, investigating the temperature dependent, weight specific, and linear growth rates of sea- and freshwater resident fish are required before this matter can be suitably resolved.

CHAPTER SEVEN

PRECOCIOUS, UNDERYEARLING CHINOOK SALMON (*Oncorhynchus tshawytscha* Walbaum) PARR: NOTES ON LABORATORY REARED FISH IN FRESH AND SEA WATER



CHAPTER SEVEN

PRECOCIOUS, UNDERYEARLING CHINOOK SALMON (*Oncorhynchus tshawytscha* Walbaum) PARR: NOTES ON LABORATORY REARED FISH IN FRESH AND SEA WATER

INTRODUCTION

Precocity, the early maturation of otherwise juvenile individuals has been variously observed in many salmonid species. For the purpose of this chapter, the description of precocity refers to underyearling parr (*i.e.* freshwater resident fish). Precocious maturation, as defined, has been observed most frequently in the parr of Atlantic salmon, *Salmo salar* (Thorpe and Morgan, 1980; Thorpe *et al.*, 1983; Baglinière and Maisse, 1985; Saunders and Schom, 1985; Thorpe, 1986a, 1987b, 1989). Dellefors and Faremo (1989) have described precocious parr in the brown trout, *S. trutta*, and it is known to have occurred in some *Salvelinus* species (Thorpe, 1986a, 1987b). Precocious parr are also relatively common the genus (*Oncorhynchus*) having been described in the parr of chinook (*O. tshawytscha*), sockeye (*O. nerka*), masu (*O. masou*), and amago (*O. rhodurus*) salmon, and in rainbow trout (*O. mykiss*, Rutter, 1904; Rich, 1920; Ricker, 1938; Robertson, 1957; Gebhards, 1960; Flain, 1970; Nævdal *et al.*, 1981; Hard *et al.*, 1985; Taylor, 1989; Kato, 1991 and references cited therein).

In the wild, precocious maturation of young freshwater fish is almost exclusively restricted to males ('dwarf males', Leyzerovich, 1973; Mitans, 1973) , and to the parr stage of development (*i.e.* prior to the development of hypo-osmoregulatory ability and the transformation to the migratory smolt stage), although exceptions have been recorded (Hindaur and Nordland, 1989; O'Connell and Gibson, 1989). Although the axiom that all Pacific salmon die post spawning stands for sea-run adults, precociously maturing chinook male parr may recondition, post spawning, and migrate to sea as yearling or two year old smolts (Rich, 1920; Robertson, 1957; Flain, 1970; Healey 1991). Atlantic salmon which had previously matured in the parr stage have demonstrated good growth at sea (Skilbrei, 1990).

There has been much recent interest into the factors that cause the onset of precocious maturation in parr (Thorpe, 1986a, 1987b; Adams and Thorpe, 1989; Hansen *et al.*, 1989b; Rowe and Thorpe, 1990a,b; Järvi *et al.*, 1991; Rowe *et al.*, 1991). The general aim of salmon farmers is to produce large, market size, immature fish in as short a time as possible. However, it is now universally accepted that there is a strong positive correlation between growth rate and maturation in salmonids (Alm, 1959; Parker and Larkin, 1959; Thorpe, 1986a, 1987b, and others cited therein). Precocious maturation is detrimental as far as commercial farmers are concerned, as energy derived from feed is directed from somatic growth toward gonadal development. Furthermore, the development of the primary and secondary sex characteristics of maturing fish (growth of the kype and large, 'canine' teeth, and the deterioration of both the external appearance and the quality of the flesh) lower the price at the point of sale. Maturation

rates of Atlantic salmon parr and post-smolts have been reduced following restriction of feed at certain 'critical' times (Rowe and Thorpe, 1990b; Thorpe *et al.*, 1990a).

Herein lies a paradox: fastest somatic growth and large size is achieved with high feeding rates; however, fast growth and attainment of large size is inexorably linked to maturation by processes that are largely unknown (Alm, 1959; Parker and Larkin, 1959; Thorpe, 1986a, 1987b; Rowe *et al.*, 1991). There has been much debate as to the regulators of maturation. Scientists on both sides of the Atlantic have discussed a 'two-threshold hypothesis' regarding critical sizes for smolting and precociously maturing juvenile Atlantic salmon (Bailey *et al.*, 1980; Thorpe and Morgan, 1980; Thorpe *et al.*, 1980). They argue that the threshold size for smolting is less than that for precocious maturation. Therefore, populations with relatively small yearling (1+) smolts would not have precocious underyearling (0+) males, whereas in populations with relatively large 1+ smolts, precocious 0+ males would occur. Both groups produced evidence in support of the hypothesis, as has subsequent work by others (Saunders *et al.*, 1982; Baglinière and Maisse, 1985).

Body size by itself however, is not enough to cause developmental change. Both processes require environmental '*zeitgebers*' (cues, prompts) at 'critical time windows' (Boeuf and Harache, 1982) in order that the developmental conversions (Smith-Gill, 1983) may be initiated. In other words, the size thresholds must be attained before, or during, a specific time period, in order that the zeitgebers can induce the neuro-endocrine system at that time, and thereby influence the developmental route (Thorpe, 1987b). Photoperiod has been proposed as the principal zeitgeber for inducing developmental change in fish (Eriksson and Lundqvist, 1982; Thorpe, 1986b, 1987a,b). Net energy balance, and dynamic hormone kinetics involved in the processing and storage of surplus energy are considered as the internal modulators of developmental conversion (Thorpe, 1987a).

Onset of maturation in both wild and 'ocean ranched' hatchery stocks, is timed such that the fish become 'ripe' during the natural spawning period - when the fish are on the spawning grounds. The changes associated with maturation are many and take time to develop fully. It follows therefore that there are certain periods, or intervals, when a 'decision' to mature for the next spawning season is taken. How this is determined by individual fish is largely unknown, although for juvenile Atlantic salmon to mature as 0+ parr, the 'decision' must be made soon after emergence (Bailey *et al.*, 1980). The decision to mature as a 1+ parr is thought to occur later, toward the end of the first summer of life (Thorpe *et al.*, 1980). It has been shown that the rate of acquisition and storage of fat, at certain 'critical' times, is an important factor in the precocious maturation of Atlantic salmon parr (Rowe *et al.*, 1991).

Depending on the salmon stock (Leyzerovich, 1973), precocious maturation of parr is not usually referred to as the 'normal' course of growth and development. Moreover, increases in the blood titres of the 'sex' steroid hormones and gonadal development, disrupts the otherwise typical increase in hypo-osmoregulatory ability and smolt transformation (Lundqvist and Fridberg, 1982; Aida *et al.*, 1984; Ikuta *et al.*, 1985, 1987; McCormick and Naiman, 1985; Nagahama, 1985; Miwa and Inui, 1986; Lundqvist *et al.*, 1989, 1990; Foote *et al.*, 1991).

Hatcheries providing smolts for stock enhancement or ocean ranching programs, aim to produce pre-adapted seawater tolerant fish for certain critical periods, most often corresponding to the natural downstream migration of wild smolts. Evropeizeva (1960) and Thorpe (1986a, 1987b) proposed that sexual maturation in the parr stage, and parr-smolt transformation were 'biologically opposite processes' and 'mutually inhibitory' respectively. Parr-smolt transformation confers hypo-osmoregulatory abilities that lead to a commitment to a period of life at sea, whereas the act of reproduction in salmonids necessarily requires a freshwater habitat.

Gilbert (1913) documented age at maturity for all species of North American Pacific salmon. He concluded that chinook (king) salmon predominantly mature in their fourth year, although the range (for males only) was from underyearling (parr) to 7 or 8 year old fish. Age structure at maturity in Californian chinook (the ancestral stock that was established in New Zealand) was discussed by Clark (1929). Spawners were predominantly four year old fish (44-49%), next common were five (24-41%), three (12-22%), and six (0.7-4%) year old fish. Two year old spawners were rare (0.2-2%) during the 1919 and 1921 seasons reported by Clark (1929). The present day age structure of the spawning run of Californian chinook is somewhat different (Kope, 1987; Dr FW Fisher, 1992, personal communication). Kope (1987) provided figures to indicate that three year old fish are now the most abundant age class (59.8%), followed by two (23.2%), four (16.9%), and five (0.1%) year old fish. The average age at spawning for Californian chinook has therefore decreased by twelve months over the course of the last 60-70 years. The factors likely to have caused these changes have been discussed in detail by Ricker (1981).

The current age structure of Californian chinook on the spawning grounds is similar to that reported for New Zealand chinook. McDowall (1990b) documented the age distribution of chinook spawners in New Zealand. Three year old fish have always predominated in the population (70%), with four year old fish the next most common (17%), and two year old fish making up the rest (13%). Five year old chinook have never been common in this country and make up less than one percent of the spawning population of any given year. Fish older than five years have not been recorded in New Zealand (Finlay, 1972; McDowall, 1990b; Mr M Flain, personal communication, 1991).

This chapter describes the occurrence of two laboratory reared, precocious male, underyearling, fall chinook salmon, one recovered from freshwater, the other from seawater. The fish were siblings from crosses of sea-run adult chinook salmon, and had been reared at a hatchery for about six months prior to transfer to laboratory conditions. The maturing freshwater fish was approximately eight months old when dissected, the seawater fish was nearly a month older. This is the first description of precocious maturation in a seawater-adapted, underyearling salmonid.

MATERIALS AND METHODS

Chinook salmon were obtained from the Ministry of Agriculture and Fisheries' (MAF) Glenariffe Hatchery, and transported to the Zoology Department (hereafter the Department). The fish were held in 60-80 litre glass tanks and were used in separate growth experiments (one fish was reared in freshwater during the final growth experiment of season 3 (*see* CHAPTER 3) and the other was transferred to seawater during the penultimate seawater transfer and growth experiment (*see* CHAPTER 6)). Growth rates were determined from routine measurements. The fish were lightly anaesthetized, and measurements of fork and standard length, length of the caudal peduncle, and body weight were made on several occasions during the period of the growth investigations.

Fulton's condition factor, $100 \times W/L^3$ (where W is the gram weight, and L is fork length in centimetres), was calculated for each fish at every measurement. Linear growth rate (LGR), $[L_f - L_i]/t$ (where L_f and L_i are the final and initial fork lengths (mm) respectively, and t is the duration of the growth period in days), and specific growth rate (SGR), $[[\ln(W_f) - \ln(W_i)]/t] \times 100$ (where $\ln(W_f)$ and $\ln(W_i)$ are the natural logarithms of the final and initial wet weights (g) respectively, and t is the duration of the growth period in days) were calculated for each fish at each remeasurement. Indices of standard length and caudal peduncle length were calculated as a proportion of fork length.

At the conclusion of the experiments, the fish were killed by overanaesthesia, and measured. The tail was severed and blood was collected into ammonium heparinised haematocrit micro capillaries from the caudal vasculature. The capillaries were spun for six minutes at 5000 *g*, and the blood haematocrit determined. Blood plasma was then transferred to plastic vials and snap frozen in liquid nitrogen. Plasma concentration ($mmol.l^{-1}$) of sodium and chloride, and osmolality ($mOsm.kg^{-1}$) were determined using routine methods (*see* CHAPTER 2). The fish were dissected to obtain heart, viscera and carcass weights. Cardiac and visceral indices were calculated as a proportion of total weight. Percentage water content of the whole heart, the entire viscera, and a piece of white muscle were also determined. Gill Na^+-K^+-ATPase activity was quantified for each fish following previously described techniques (Johnson *et al.*, 1977; Langdon *et al.*, 1984; Franklin, 1989). All samples were stored at -80 °C until analysis.

All data were entered into spreadsheet software for calculations and statistical analysis. Results are presented as a single statistic (*i.e.* precocious male), or the sample mean $\pm$standard error, or $\pm$95% confidence limits ($n=5$). Comparison of all the variables of the precociously mature fish with those from the group of immature, sibling fish, was performed using the Standard Error test (Sokal and Rohlf, 1981). Statistical significance between each single growth parameter of the maturing fish and the group means of immature fish were recorded when $p \leq 0.05$.

RESULTS

Growth rates recorded by the two precociously mature chinook compared to those of immature sibling fish are presented in Table 7.1 (freshwater) and Table 7.2 (seawater). In both cases, the maturing fish was initially measured as part of the group against which it was compared. However, as each fish was individually branded, it was possible to treat the measurements for each maturing fish separately from the rest of the group.

At the start of each separate growth experiment, it should be noted that both the maturing fish were larger (fork length and body weight) than the average size of the immature fish. The linear growth rate (LGR) of the fish that matured in freshwater (Table 7.1) was slower than that of the immature fish. In contrast, specific growth rate (SGR) was equivalent between the fish. However, due to the relatively poor LGR, condition factor (CF) increased markedly in the maturing fish. Whilst the difference in body weight was kept throughout the experiment, the higher LGR of immature fish caused the initial difference in fork length to disappear. The maturing fish was visibly different from the other fish as it had a deeper body and a distended belly. The back of the fish became a deeper green, and the colouration of the spots intensified. These differences became increasingly obvious during January, 1992.

Table 7.1 Growth statistics for the underyearling chinook that matured in freshwater. Comparisons of growth rates from a group of immature sibling fish are also given. The male symbol (♂) represents the mature male, and the group is given by the statistical mean symbol ($\bar{x}$). Data for the group are given as the sample mean ±standard error (n=5). Changes of condition factor, specific growth rate (SGR) and linear growth rate (LGR) were calculated from successive measurements of fork length and body weight, as per sampling date. Significant differences between each measurement for the maturing and immature fish are given by asterisks (*) when $p \leq 0.05$, single daggers (†) when $p \leq 0.01$, and by double daggers (‡), when $p \leq 0.001$.

Sample Date		Fork Length (mm)	Body Weight (g)	Condition Factor	SGR (%wt.day^{-1})	LGR (mm.day^{-1})
6/12/91	♂	111.68 ‡	16.78 †	1.20	-	-
	$\bar{x}$	107.75±0.50	14.61±0.41	1.17±0.02	-	-
23/12/91	♂	122.90 *	26.47 †	1.43 ‡	2.68	0.66 ‡
	$\bar{x}$	121.28±0.58	23.86±0.45	1.34±0.01	2.89±0.12	0.79±0.01
10/1/92	♂	128.68	31.69 †	1.49 ‡	1.00	0.32 *
	$\bar{x}$	129.74±1.16	28.62±0.65	1.31±0.01	1.12±0.11	0.50±0.07

Slightly different changes occurred in the parameters of growth recorded from the underyearling chinook that matured in seawater (Table 7.2 and *see* Figure 7.1 on page 131). Although the maturing fish was larger (fork length and body weight) than the average size of immature sibling fish upon seawater transfer, it exhibited relatively poor SGR and LGR over the following two months and was smaller at dissection. Nevertheless, CF of the maturing fish

steadily increased and was significantly higher at the completion of the experiment. Although the maturing fish had a much darker appearance than its immature siblings throughout the experiment, the distended belly, typical of maturing fish, was only apparent during 1992. The colour difference was most noticeable immediately after transfer. Indeed, during the seawater challenge test, the animal lost 1% of its length, and almost 12% of its weight. A colour photocopy of this fish is presented in Figure 7.1.

Table 7.2 Growth statistics for the underyearling chinook that matured in seawater. Comparisons of growth rates from a group of immature sibling fish are also given. Changes of condition factor, specific growth rate (SGR) and linear growth rate (LGR) were calculated from successive measurements of length and weight, as per sampling date. The measurements on 25 and 27 November 1991 represent the fish before and after the 48 hour seawater challenge test respectively. Data for the immature group are given as the sample mean ±standard error (n=5). For an explanation of the symbols used refer to the legend in Table 7.1.

Sample Date		Fork Length (mm)	Body Weight (g)	Condition Factor	SGR (%wt.day^{-1})	LGR (mm.day^{-1})
25/11/91	♂	97.32	9.92	1.08	-	-
	$\bar{x}$	94.46±1.46	8.81±0.55	1.04±0.02	-	-
27/11/91	♂	96.28	8.74	0.98	-	-
	$\bar{x}$	93.94±1.68	8.53±0.57	1.02±0.02	-	-
23/12/92	♂	100.34	11.87	1.17	1.18 *	0.16 †
	$\bar{x}$	104.12±1.97	13.83±1.30	1.21±0.05	1.82±0.21	0.39±0.04
13/1/92	♂	103.12	13.85	1.26 *	0.73	0.13 *
	$\bar{x}$	109.12±2.55	15.65±1.29	1.19±0.02	0.58±0.09	0.24±0.04
3/2/92	♂	113.36 *	19.82 *	1.36 *	1.71	0.49 *
	$\bar{x}$	122.94±3.22	23.25±1.31	1.25±0.04	1.94±0.25	0.66±0.05

Morphologically, there were little differences between the fish with respect to maturity (Table 7.3). Only the indices of standard length and caudal peduncle length of the seawater maturing fish were significantly smaller than those from the immature seawater group. Both length indices recorded from the seawater maturing fish resembled the values for freshwater resident fish. No differences were observed between maturing and immature fish in terms of visceral and cardiac indices. Although the gonadal indices differed in absolute amount between the two maturing fish, this was probably a reflection of the length of time that had elapsed since the onset of the maturation process.

Post mortem differences of gill Na^+-K^+-ATPase activity, blood haematocrit, plasma osmolality, and plasma concentrations of sodium and chloride were compared between the two maturing fish and their respective immature sibling groups (Table 7.4). No significant differences ($p>0.05$) were found between any of the physiological variables for the freshwater

fish. However, the maturing seawater fish had a significantly lower gill Na^+-K^+-ATPase activity ($p<0.05$) compared to that recorded for immature siblings. In addition, the blood haematocrit was significantly elevated ($p<0.01$) in the maturing fish. No differences were observed for levels of plasma osmolality and the plasma concentrations of sodium and chloride.

Table 7.3 Comparison of various morphological indices between the solitary maturing male and the group of immature siblings from both the freshwater and seawater experiments. The indices of standard length and caudal peduncle length are expressed as a proportion of the fork length. Visceral, cardiac, and gonadal indices are given as a proportion of body weight. Data for the immature group are given as the sample mean ±standard error ($n=5$). For an explanation of the symbols used refer to the legend in Table 7.1.

Group and Sample Date		Standard Length Index (%)	Caudal Peduncle Index (%)	Visceral Index (%)	Cardiac Index (%)	Gonadal Index (%)
Freshwater	♂	89.84	12.43	15.54	0.11	6.32
10/1/92	$\bar{x}$	89.85±0.41	12.87±0.41	15.07±1.34	0.11±0.00	-
Seawater	♂	89.43 *	12.44 *	15.96	0.11	7.27
3/2/92	$\bar{x}$	90.35±0.35	13.16±0.28	15.78±1.77	0.12±0.00	-

Table 7.4 Comparison of various physiological parameters between the solitary maturing male and the group of immature siblings from both the freshwater and seawater experiments. Data for the immature group are given as the sample mean ±standard error ($n=5$). For an explanation of the symbols used refer to the legend in Table 7.1.

Group and Sample Date		Na^+-K^+-ATPase (μmol P_i.mg protein^{-1}.h^{-1})	Haematocrit	Osmolality (mOsm.kg^{-1})	$[Sodium]_{pl}$ (mmol.l^{-1})	$[Chloride]_{pl}$ (mmol.l^{-1})
Freshwater	♂	6.31	36.31	308	145	118
10/1/92	$\bar{x}$	6.95±0.50	36.19±0.76	311±3	145±1	119±1
Seawater	♂	9.66 *	43.80 †	337	159	134
3/2/92	$\bar{x}$	12.95±0.65	38.33±1.07	342±5	161±1	135±4

DISCUSSION

Precocious parr ('dwarf males') have previously been recorded in populations of New Zealand chinook salmon (Flain, 1970). The fish were selected at random, measured, and placed into tanks for growth and growth rate studies. The fish were discovered to be maturing upon dissection, at the end of the experimental growth period. Precocious maturation of underyearling fish used in this study was not common; only two fish (out of the 113 fish that reached a similar size) exhibited the phenomenon. Flain (1970) reported a high incidence (19.0-29.2%) of maturation in two yearling populations of wild chinook in this country. Earlier

reports gave the incidence of precocious maturation of yearling chinook that are more in line with the studies undertaken here (1.1%; New Zealand Marine Department, 1936).

Although morphological changes were discernable between the maturing and immature fish, they were only evident in the latter stages of the experiments. Both maturing chinook developed significantly higher condition factors (CF; Tables 7.1 and 7.2). Increase in CF was directly attributable to the additional weight of the developing testes as the magnitude of the visceral index did not change in the maturing fish (Table 7.3). The stomach and intestines of both fish were full upon dissection. This is in contrast to the maturation process of 'adult' salmon. Such fish cease feeding, with gonadal development being derived from the remarkable process whereby the somatic muscle and abdominal fat stores are metabolised, and the nutrients

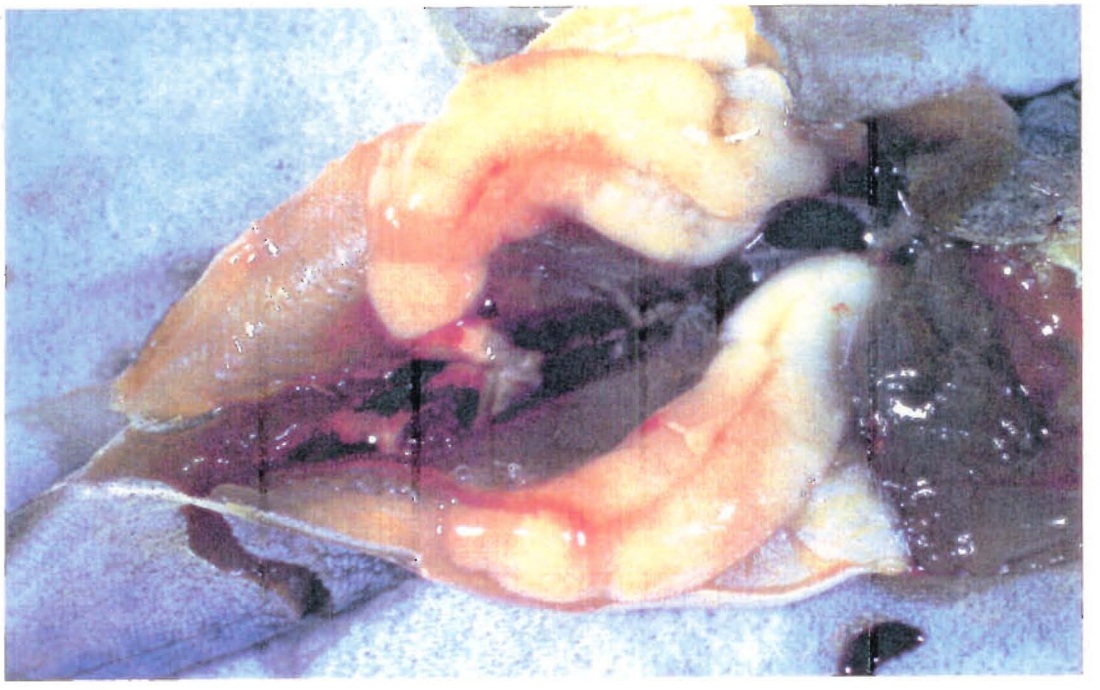


Figure 7.1 Photocopy enhanced positive of the underyearling chinook fingerling (113.36 mm fork length, and 19.82 g body weight) that matured in seawater showing the developed testes. The testes weighed 1.44 g upon dissection, accounting for 7.27% (*i.e.* the gonadal index) of the total body weight. Photography by Mr D Shaw-Brown, senior university photographer.

incorporated into the reproductive tissue. Though the specific growth rates (SGR) of both maturing fish were generally similar to those of immature individuals, their linear growth rates (LGR) were significantly retarded, and hence substantial increases in CF were recorded (Tables 7.1 and 7.2). Despite these differences, predictive indicators of precocious maturity were not obvious. Rather interestingly, the standard and caudal peduncle indices of the fish that matured in seawater were significantly less than in immature seawater fish. Furthermore, both indices were similar to those observed in freshwater resident fish, and it would appear that this fish had retained these freshwater traits.

Both of the maturing fish were larger than the average size of their immature siblings at the start of the experiments (Tables 7.1 and 7.2). Bailey and associates (1980) speculated that in order for an Atlantic salmon, *Salmo salar*, to mature as a yearling parr, the 'decision' to do so would have to be taken soon after emergence. Whether the two maturing fish reported here were relatively large upon hatching and subsequently maintained their size advantage or whether they grew at a faster rate than their immature siblings, is not known. Decreased somatic growth, during precocious maturation of Atlantic salmon, has been recorded previously (Thorpe and Morgan, 1980; Saunders *et al.*, 1982; Thorpe *et al.*, 1983; Rowe and Thorpe, 1990a).

The fish that matured in freshwater was always significantly heavier than the group of immature siblings (Table 7.1). Although fork length increased with time, LGR was relatively slower than the immature fish, and as a result no difference in terms of length was evident at the end of the experiment (Table 7.1). Initially, the growth rate of the seawater maturing fish was significantly less than recorded by immature siblings (Table 7.2). Whilst the SGR 'recovered', and increased to a similar rate observed in the immature fish, LGR was depressed throughout the growth experiment. Furthermore, despite being larger than average at the start of the experiment, 'poor' growth caused the precocious fish to be significantly smaller (length and weight) than its immature siblings at the conclusion of the experimental growth period.

As SGR and LGR were initially lowered, the growth data for the seawater fish are in agreement with Thorpe's (1986a, 1987b) hypothesis that parr-smolt transformation and maturation are mutually inhibitory. It is of course not possible to say whether this seawater fish had initiated the maturation process prior to being transferred to seawater (an event which it would not have 'foreseen'). However, the weight and length decrease data during the seawater challenge test would suggest that it had. The dual, energetic cost of maintaining homeostasis in the saline environment, and developing gonadal tissue, may in part explain the relatively poor somatic growth of this animal. Gill Na^+-K^+-ATPase activity was significantly lower in the maturing fish (Table 7.4). Furthermore, blood haematocrit was significantly higher, indicating either that the fish was in a 'stressed' state, or that it was unable to osmoregulate optimally in the marine environment. However, no differences in plasma electrolyte concentration or plasma osmolality were apparent between either maturing fish and their respective immature siblings.

Precocious maturation in a seawater adapted, underyearling chinook salmon has not been recorded previously. Indeed, Foote and associates (1991) described that parr-smolt transformation of chinook salmon inhibited by precocious maturation. Sutterlin and co-authors (1978) have described precocious maturation in Atlantic salmon following seawater transfer. Yearling smolts were transferred to seawater in the spring, and after rearing over the summer, up to 16% population (males and females) had matured. Atlantic salmon typically require at least one 'sea-winter' prior to the development of sexual maturation (Sutterlin *et al.*, 1978).

The seawater maturing fish in this study was approximately eight months old at dissection and had large testes accounting for 7.27% of the body weight. MacKinnon and Donaldson (1976) reported that nine (of 200) pink salmon (*Oncorhynchus gorbuscha*) were induced to mature precociously as yearlings following accelerated growth in warm seawater (12 °C; the

'normal' water temperature was not given). The characteristic life cycle of pink salmon is one that follows a rigid two-year cycle, terminating in sexual maturation, spawning, and death (Heard, 1991). The gonads of the precocious pink salmon accounted for 6.77±1.40% (mean±SE) of the body weight (MacKinnon and Donaldson, 1976). The experiment on the pink salmon was concluded at the natural spawning time of the stock, and post-mortem inspection showed that milt could be expressed from six of the maturing fish. Furthermore, histological analysis of the testes indicated that the other three fish were approaching full maturity.

Milt could not be expressed from either of the maturing fish in this study. However, as the peak spawning period for New Zealand chinook is between late April and early May, it is perhaps not surprising that the maturing fish were not ripe (the freshwater fish was killed in early January, the seawater fish in early February). Saunders and Henderson (1965) reported gonadal indices for precocious Atlantic salmon 'post-smolts' realising up to 5.37% of the body weight (16-19 month old fish with 4-7 months in seawater). Taylor (1989) documented gonadal indices from two separate, laboratory-reared populations of chinook salmon (14 month old fish). The testes weights were 5.48±0.39%, and 5.78±0.53% of body weight for the Bowron River, and Slim Creek populations respectively. Variation in the magnitude of the gonadal index between the studies is probably due to the timing of post-mortem analyses with respect to the developmental stage of the testes. Funk and Donaldson (1972) estimated the degree of maturity in precocious pink salmon on the basis of six distinct histological stages that occur during testicular development. Testicular growth is characterised by rapid growth of the testes to a maximum size. This size is reached prior to full maturation of spermatozoa and the presence of spermatozoa in the sperm duct.

Part of the studies undertaken in this thesis involved the acceleration of growth rate by rearing underyearling chinook fry in 'warm' water during season 3 (13-15 °C in the Department, as opposed to 7-11 °C at Glenariffe hatchery). In total, some forty fish were reared in this way and were used in various growth experiments (*see* CHAPTER 6). The last sample of fish transported from Glenariffe in season 3 had a fork length of 107.79±2.5 mm (mean±95%CL), and weighed 14.94±1.17 g (6 December 1991). The accelerated growth fish were 137.54±8.31 mm long, and weighed 35.17±6.70 g at the same time (17 December 1991). Condition factor of the accelerated group (1.34±0.02) was also greater than the Glenariffe reared fish (1.19±0.03).

Therefore, linear and specific growth rates were 0.55 mm.day^{-1} and 2.76 %body weight.day^{-1} for accelerated growth fish, and 0.46 mm.day^{-1} and 2.57 %body weight.day^{-1} for Glenariffe reared fish. None of the accelerated growth fish matured during extended rearing (up to seven months) in the Department. Both of the maturing fish were reared for shorter periods within the Department (less than nine weeks). It is not possible to say whether either fish had started to mature prior to relocation. 'Trophic opportunity' in the form of improved rearing conditions (lower fish density, warmer water, and higher individual feeding rates) was likely to have promoted faster individual growth (Thorpe, 1987b). Both fish were transported during the period when sibling fish were undergoing parr-smolt transformation (*see* CHAPTER 5).

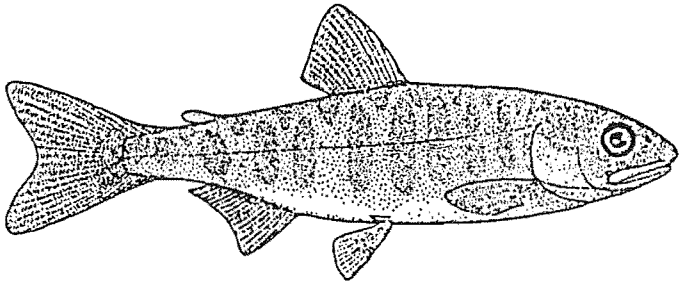
Thorpe (1987b) argues "*The contention, then, is that the developmental route taken by the fish - either to smolt or to mature - depends on the trophic opportunities available to them at the seasonally critical times.*" Increasing photoperiod may synchronise the onset of parr-smolt transformation, and decreasing photoperiod may synchronise maturation in salmonids (Eriksson and Lundqvist, 1982). Research has demonstrated that chinook salmon do not necessarily follow these generalisations. Clarke and associates (1981) found no evidence for the photoperiodic regulation of growth or osmoregulatory changes in their studies on underyearling, fall chinook salmon. Similar results regarding an independence of photoperiod during growth and development of juvenile, spring chinook salmon have also been reported (Ewing *et al.*, 1979).

It may be therefore, that long term rearing under the unchanging photoperiod (12L:12D) experienced in the laboratory would have had no effect on the occurrence of parr-smolt transformation, but would have lessened the likelihood of maturation. Fish transferred from Glenariffe at the end of November, early December would have experienced a sudden decrease in day length (approximately 15L:9D, natural photoperiod for late November, early December, with increasing day length). Provided the fish were physiologically sensitive to such an environmental change upon arrival at the Department, and in consequence of the increased 'trophic opportunity' available during laboratory rearing, the process of maturation may have been induced in these two precocious fish. Should this be the case, the seawater maturing fish developed 1.44 g of gonadal tissue in approximately 70 days, out of a total weight gain of 9.90 g (14.5% of the weight gained). Similarly, the freshwater fish gained a total weight of 14.91 g in 35 days, of which 1.97 g were developing testes (13.21% of the entire weight gain).

Both precocious 'parr' (a misnomer for the fish transferred to seawater) were considerably younger than fish detailed in previous reports. Both were likely to become fully mature as yearling fish during the natural spawning period. Salmonids are opportunistic, phenotypically plastic generalists, and exhibit highly variable life-history strategies (variations are often distinguishable within species). Discrete spawning stocks (Ricker, 1972) however, have evolved within the confines of narrow environmental niches, and therefore salmonids can also be termed as being 'specialists'. The chinook salmon probably exhibits the widest variety of life-history strategies. That an underyearling chinook underwent the processes of parr-smolt transformation *and* precocious maturation within a few months of each other serves to add to the abundant literature regarding the phenotypic plasticity demonstrated by this species.

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSIONS



CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSIONS

GROWTH AND GROWTH RATE INVESTIGATIONS

Chinook salmon growth

The growth rate of chinook salmon (*Oncorhynchus tshawytscha*) was examined throughout development from 'zip-up' fry to 'post-smolt' (nine month old) fish. Specific growth rate (SGR) was fastest during the pre-smolt, fry stage, whereas linear growth rate (LGR) was highest during the period of parr-smolt transformation (Figure 3.3). 'Zip-up' fry grew relatively slowly (particularly with regard to LGR) compared to the fully buttoned-up fry, probably due to physiological changes associated with complete yolk-sac absorption and the switch to exogenous nutrition. At this stage of development, SGR was high relative to LGR, and thereby brought about the morphological transformation of the full bodied, fusiform fingerling from the lean, elongated 'zip-up' fry. SGR declined prior to the period of parr-smolt transformation, whereas LGR increased. This reciprocal situation of high LGR and slower SGR would lead to relative body elongation at this time, yielding the characteristically more slender and 'streamlined' smolts (Figure 3.3). Condition factor of the laboratory reared fish did not decline over the period of parr-smolt transformation, as has been reported in 'wild' (*i.e.* naturally spawned) smolts (Vanstone and Markert, 1968; Fessler and Wagner, 1969; Virtanen, 1987). However, salmonids reared under laboratory conditions do not necessarily demonstrate a decrease in condition at the smolt stage (Folmar and Dickhoff, 1980; Gorbman *et al.*, 1982; Zaugg, 1982b,c; Langdon, 1985; Schreck *et al.*, 1985; Virtanen, 1987).

Slight modifications of body morphology however were observed during the period of parr-smolt transformation (increases in the proportion of caudal peduncle length and standard length, relative to fork length, Figure 3.4). Reported previously in Atlantic salmon (Nikolskii *et al.*, 1947), coho and chinook salmon (Winans, 1984; Winans and Nishioka, 1984; Taylor and McPhail, 1985b), such changes in 'smolts' are pre-adaptive for an increased pelagic life, whereas the retention of a short, broad peduncle "*makes for naturally good swimming when living in a rapid current*" (Nikolskii *et al.*, 1947) typical of the riverine environment. Data from seawater resident fish (Table 6.2) however, indicated that the length of the caudal peduncle and the standard length were apparently unaffected by salinity, although longer rearing may have caused a change. Further study, and the measurement of many other morphometric characters using more advanced digitizing techniques (Winans, 1984) may well provide further information regarding morphological changes associated with early growth and development of chinook.

Gill Na^+-K^+-ATPase increased steadily during spring peaking around October to December (late spring, early summer; Figures 3.7 and 3.9), which is the natural period of parr-smolt transformation for this species (Unwin, 1986; McDowall, 1990b; Taylor, 1990c; Healey, 1991), and which coincided with survival of fish transferred to seawater

(Figures 5.1 and 5.2). During the period of parr-smolt transformation and seawater survival, the chinook grew from approximately 65 mm (fork length) and 2.5 g (body weight) to around 104 mm and 12 g. Clarke (1982) argued that chinook must reach a minimum size of five grams before they can withstand a transfer to seawater. The results presented here are in good agreement with this earlier work. Percentage water content of the white muscle, the heart and the viscera decreased during freshwater growth and development, the greatest decrease being observed in the viscera (Figures 3.6 and 6.6).

Blood haematocrit, plasma osmolality, and plasma sodium and chloride concentration fluctuated during growth and development. Resting values of untrained fish were approximately 270-310 $mOsm.kg^{-1}$, 140-150 $mmol.l^{-1}$ and 120-130 $mmol.l^{-1}$ respectively (Figures 3.7 and 3.9, Table 6.3), which are within the normal physiological range of freshwater resident salmonids (Wedemeyer *et al.*, 1980; Folmar and Dickhoff, 1981; Hoar, 1988). Although training caused the three plasma variables to decrease slightly, a variable response was observed with haematocrit (Figures 3.8 and 3.10). Training may have caused a stress related increase in haematocrit, and ion flux imbalances, which have previously been recorded by Wood and Randall (1973a,b,c) in their studies of extended exercise in rainbow trout, *Oncorhynchus mykiss*, and by Virtanen and Forsman (1987) in their experiments on the swimming ability of wild Atlantic salmon, *Salmo salar*, parr and smolts.

Exercise training

Improved growth of a number of salmonid species, following low intensity exercise training, has been reported previously (Davison and Goldspink, 1977; Kuipers, 1982; Nahhas *et al.*, 1982b; Leon, 1986; East and Magnan, 1987; Houlihan and Laurent, 1987; Totland *et al.*, 1987; Christiansen and Jobling, 1990; Farrell *et al.*, 1990). Other papers have investigated the problem but have not found any significant effect of training on growth rate (Shaw *et al.*, 1975b; Greer-Walker and Emerson, 1978; Christiansen *et al.*, 1989), and at least one paper has been published recording similar results to those reported here, *i.e.* that exercise training caused a significant reduction of growth rate in trained fish, relative to the growth rate of untrained fish (Farrell *et al.*, 1991).

The growth of underyearling chinook salmon was clearly reduced by exercise training in this study (Figures 3.2 and 3.3). Three differing training regimes were used during the course of the investigation, with all three effecting reduced growth in trained fish relative to that attained by untrained fish in still water. Significant growth retardation was observed in trained fish after only 10-13 days of training. Untrained hatchery chinook grew at up to four (SGR) and five (LGR) times the rate of trained fish during the 15 separate growth *versus* exercise training trials (Figures 3.2 and 3.3). Moreover, the effect was observed in all developmental stages from 'zip-up' fry to 'post-smolt' fish, and in a trial involving wild chinook (Tables 3.1). The growth of hatchery reared sockeye salmon was unaffected by training (Table 3.2).

It would appear that exercise training can have one of three effects on the growth of salmonids; enhancement of growth, no effect, or growth retardation. Furthermore, the effect

of exercise training on growth appears to be related to the *natural* growth rate of any given species. Training has tended to promote the growth of the slower growing salmonids (*Salmo* and *Salvelinus* species; Kuipers, 1982; Leon, 1986; East and Magnan, 1987; Totland *et al.*, 1987; Christiansen and Jobling, 1989; Christiansen *et al.*, 1990) and yet has reduced the growth of the naturally faster growing species (*Oncorhynchus* species; Farrell *et al.*, 1991; and this study). There are, of course, contradictory results, particularly regarding work on rainbow trout (Greer-Walker and Emerson, 1978; Nahhas *et al.*, 1982a,b; Houlihan and Laurent, 1982; Farrell *et al.*, 1990, 1991). Additionally, the conflicting data are in large part due to the differing exercise training regimes imposed in each separate study.

Kerr (1971) and McNeish and Hatch (1978) observed that salmonids undertake intermittent and periodic bouts spontaneous activity, and tend to refrain from sustained and continuous swimming. It might be therefore that training acted as a 'stressor' on the groups of trained fish used in this study, and thereby reduced their growth rate. The general stress response of salmonids elevates plasma titres of the corticosteroids (Pickering, 1981; Schreck, 1982b; Redding and Schreck, 1983; Franklin, 1989; Franklin *et al.*, 1992). Enforced swimming (at speeds equivalent to those used here) for two hours has caused significant elevation of plasma cortisol in rainbow trout (Zelink and Goldspink, 1981). Furthermore, training consistently lowered gill Na^+-K^+-ATPase activity, particularly during the period of parr-smolt transformation (Figures 3.8 and 3.10). Furthermore, cortisol has been shown to paradoxically enhance (Richman *et al.*, 1985; Richman and Zaugg, 1987; McCormick and Bern, 1989; Madsen, 1990a,b,c; Bisbal and Specker, 1991) and reduce (Langdon *et al.*, 1984; Redding *et al.*, 1984) the hypo-osmoregulatory ability of salmonids. Additionally, seawater survival was markedly lower in Atlantic salmon smolts that were 'stressed' at the time of transfer (Järvi, 1989).

Gill Na^+-K^+-ATPase activity has been associated with initiating downstream migration and seawater survival in many salmonids (Zaugg and McLain, 1972; Zaugg and Wagner, 1973; Giles and Vanstone, 1976a; Johnson *et al.*, 1977; Ewing *et al.*, 1979; Buckman and Ewing, 1982; Franklin, 1989; Zaugg, 1989; Zaugg and Beckman, 1990). Enzyme activity 'normally' increases prior to seawater entry, and often during the downstream migration of smolts (Ewing, Fustish *et al.*, 1980; Langdon and Thorpe, 1985; Zaugg *et al.*, 1985). Some research however has reported that increased gill Na^+-K^+-ATPase activity is not a prerequisite for commencing seaward movement (Ewing, Fustish *et al.*, 1980). Additionally, the literature is divided regarding whether or not maximal returns of salmon released in ocean ranching programmes can be predicted by peak activity of this enzyme or by other physiological indicators of 'full smolt condition' (*e.g.* plasma thyroxine ($[T_4]_{pl}$) peaks, gill mitochondrial succinic dehydrogenase (SDH) activity, 'energy' stores, condition factor, body silvering, *etc.*). Some workers have found no correlation (Ewing, Fustish *et al.*, 1980; Bilton *et al.*, 1982; Ewing and Birks, 1982; Ewing *et al.*, 1985), whilst others report strong correlation (Zaugg and Wagner, 1973; Whale and Zaugg, 1982; Soivio and Virtanen, 1985; Zaugg, 1989) of adult return rate with one or other of the physiological parameters of parr-smolt transformation, measured prior to smolt releases.

Two underyearling chinook precociously matured in this study. Whilst they were visibly different from non-maturing siblings, only condition factor was significantly elevated in these fish, and by the time the difference was noted, the processes of maturation was probably too far developed to be curtailed by feed restriction. One of the two 'precocious parr' matured in seawater within four months of transfer. This is the first such recording of maturation in an underyearling, seawater resident chinook. Interestingly the developmental processes of parr-smolt transformation and maturation are considered as being 'mutually inhibitory' (Evropeizeva, 1960; Thorpe, 1986a, 1987b). Whereas parr-smolt transformation accords hypo-osmoregulatory abilities that enable a period of marine growth, the spawning act of salmonids requires a freshwater environment.

Accelerated rearing

The growth of the underyearling chinook was greatly accelerated when reared in the warmer water of the laboratory (Figure 6.1). Enhanced growth was particularly evident in fish that were reared in the Department on a long-term basis (Figure 6.2). These results are in accordance with previously published research (Donaldson and Brannon, 1975; Brannon *et al.*, 1982; Ísaksson, 1985; Kazakov *et al.*, 1988). The reported effects of accelerated growth in 'warmer' water on subsequent seawater survival and growth performance indicate that it can be disruptive (Zaugg *et al.*, 1972; Zaugg and McLain, 1976; Hoar, 1988; Soivio *et al.*, 1988; Dickhoff, *et al.*, 1989) or beneficial (Saxton *et al.*, 1983; Ísaksson, 1985; Kazakov *et al.*, 1988; Kasahara *et al.*, 1989). Further work may be able to determine whether the timing of salt water survival and subsequent seawater growth performance of New Zealand chinook are influenced by accelerated rearing regimes. Moreover, control of feeding rates on a percentage of body weight per day basis may influence the natural oscillation, and magnitude of gill Na^+-K^+-ATPase activity. Clarke and associates (1981) considered that the parr-smolt transformation of chinook developed independently of photoperiodic influences. They argued that growth rate or size was the major determinant of hypo-osmoregulatory development in this species. Therefore, accurately controlled feeding experiments may elucidate whether growth rate and/or size influence development of seawater tolerance independently of external cues.

Seawater growth

Growth rates of seawater resident and freshwater reared fish did not differ with respect body size (Figures 6.3 and 6.4). Given the limitations of the Departmental seawater system this is a remarkable result. Salinity did vary considerably (28-38‰) over the period of examination. Such environmental fluctuations would have required the osmoregulatory mechanisms of the salmon to continually adjust their net rates of ion and water movement relative to the demands of the variable environment.

Most studies that have investigated the effect of salinity on salmonid growth have found that growth is either fastest in freshwater and decreases with increasing salinity (Saunders and Henderson, 1969; Clarke *et al.*, 1981; Nahhas *et al.*, 1982a; McKay and Gjerde, 1985; Morgan

and Iwama, 1991), or is fastest in brackish (isotonic) water (Canagaratnam, 1959; Bullivant, 1961; Otto, 1971; Kepshire and McNeil, 1972), or is generally unaffected by salinity (Shaw *et al.*, 1975b; McCormick *et al.*, 1989). Many of these studies however investigated whether the growth of fry (*i.e.* pre-smolt fish) was affected by salinity, and therefore much of the comparisons were drawn between groups of fish that would not have been altogether physiologically adapted to their environment.

The highest rates of gill Na^+-K^+-ATPase activity were recorded in seawater resident fish (Table 6.3), and indicated that the enzyme activity was some two to five times greater than the levels recorded in freshwater fish during the period of parr-smolt transformation (compare data in Table 6.3 and Figures 3.7, 3.9, 5.1, and 5.2). Gill Na^+-K^+-ATPase activities greater than 4-5 μmol P_i.mg protein^{-1}.h^{-1} were strongly correlated with seawater survival (Figures 5.1, 5.2 and 5.3). Resting levels of plasma electrolytes and plasma osmolality were higher in seawater resident, compared to freshwater reared fish (Table 6.3), but blood haematocrit was unaffected by salinity.

Water content of the viscera of seawater resident fish decreased with growth but not to the extent observed in the freshwater reared fish (Figure 6.6). Usher and associates (1991) found that the proximate condition of Atlantic salmon smolts was influenced by salinity, and related that the seawater resident fish had higher (carcass) water contents. Smith and Thorpe (1976) have provided evidence to suggest that the efficiency of nitrogen retention is greater in seawater resident fish, and that this leads to the larger size of salmonids that have spent a period of life in the ocean. Clearly the laboratory studies suggest that enhanced growth is not influenced by the marine environment and the greater availability of prey items *per se*, but is more to do with the incorporation of a more efficient intermediary metabolism with respect to nutrient (*i.e.* nitrogen) assimilation. However, the questions that arise regarding exactly how enhanced growth is mediated in the marine environment remain unanswered.

Summary of growth, and growth versus exercise investigations

In summary therefore, the exercise training regimes used in this study do not appear to be viable options for enhancing the growth of underyearling chinook salmon. Growth rates of trained fish were retarded, and their hypo-osmoregulatory ability impaired (as assessed by lowered gill Na^+-K^+-ATPase activity). Growth rate of chinook salmon was greatly accelerated in 'warm' 13-15 °C (compared to 7-11 °C) water, although it remains to be determined whether warm water rearing would disrupt the progression of parr-smolt transformation and thereby affect seawater survival. Chinook grew well in seawater, provided the fish were transferred as 'smolts' (assessed by 48 hour seawater challenge tests and gill Na^+-K^+-ATPase activity). Furthermore, 'stunting' (Clarke and Nagahama, 1977) and 'parr-reversion' (Mahnken, 1973) which are characteristic of coho salmon (*Oncorhynchus kisutch*) transferred to seawater outwith their optimal period for survival and growth, are not common of chinook.

CRITICAL SWIMMING SPEED, U_{crit}

Critical swimming speed (U_{crit}) measurements of 'zip-up' fry, fry, fingerling, smolt and 'post-smolt' chinook salmon were assessed in a Blažka-type swim-tunnel (Blažka *et al.*, 1960). The studies indicated that relative swimming speed, in terms of body lengths per second ($bl.s^{-1}$), decreased with fish size (Figure 4.1A; Wardle, 1975, 1977). Real speed U_{crit} ($cm.s^{-1}$), on the other hand, increased with increasing size (Figure 4.1B). Seawater resident fish had critical swimming speeds equivalent to freshwater siblings provided they had exhibited good growth following transfer (Figure 4.2). Moreover, there was no difference between the swimming abilities of wild and hatchery chinook (underyearling, 0+ smolts; Figure 4.3). Sockeye salmon had poor swimming abilities relative to chinook (Figure 4.4). Swimming ability of the New Zealand sockeye was comparable to that observed by Brett and associates (1958), but lower than recorded by Taylor and Foote (1991). Moreover, this later study revealed that pure bred kokanee (non-anadromous form of sockeye) had a measurably inferior swimming ability relative to pure bred sockeye of an equivalent size (Table 4.3). The decreased swimming ability of the sockeye used in this study may have resulted from their non-anadromous, lacustrine habit in New Zealand (Taylor and Foote, 1991). It would be interesting therefore to similarly compare the swimming ability of juveniles from sea-run and voluntarily landlocked chinook populations in New Zealand.

No decrease in U_{crit} performance associated with the timing parr-smolt transformation was apparent. Reductions in swimming ability have been shown in smolts of Atlantic (McCleave, 1978; Thorpe and Morgan, 1978b; Thorpe *et al.*, 1981; Virtanen and Forsman, 1987; Thorpe *et al.*, 1988), and coho salmon (Glova and McInerney, 1977; Flagg and Smith, 1982; Smith, 1982; Flagg *et al.*, 1983). Furthermore, Smith (1982) hypothesised that a reduction in swimming ability is mandatory for downstream movement of smolts, which he argues is largely a passive migration. However, the downstream migrations of pink (*Oncorhynchus gorbuscha*), and chum (*O. keta*) salmon fry, the salmonid species that naturally exhibit the strongest degree of anadromy (Rounsefell, 1958) have been rather colourfully described as being far from passive. The post-emergent fry of pink salmon were described by Pritchard (1944) to "*make a swift and vigorous migration*", and by Neave (1955) to "*travel right at the surface and create a 'bow-wave'*", and to migrate "*at a speed much greater than the current*". Wickett (1959, in Heard, 1991) calculated that pink fry migrate downstream roughly 0.5 $m.s^{-1}$ faster than the river current. Similarly, chum fry have been reported to "*swim rapidly with the current-usually more rapidly than the current*" by Hoar (1951, 1953, 1958), and by others (Neave, 1955; MacKinnon and Brett, 1955; Iwata, 1982b). Migration in both pink and chum fry is predominately a nocturnal phenomenon, and is slowed during periods of bright moonlight (Pritchard, 1944; Neave, 1955).

In contrast to the extremely anadromous pink and chum, the sockeye salmon smolt naturally migrates seaward after at least one year's growth in freshwater (Burgner, 1991). Nevertheless, Johnson and Groot (1963) and Groot (1965) stated that the seaward migration of

sockeye smolts was characterised by active and directed swimming, at close to the maximal sustained swimming speed of sockeye at the ambient water temperature (Brett *et al.*, 1958). The migration was largely nocturnal. Hartman and associates (1967) reported that sockeye smolts tend to migrate in schools, orient downstream (*i.e.* head first), and swim faster than the current. When faced with a stretch of turbulent water (such as a weir), the sockeye were observed to turn around, passing downstream, tail first.

The evidence would suggest therefore that fry outmigration (excepting pink and chum salmon) is largely directed by the water current. Fry tend to be displaced downstream during the hours of darkness following loss of orientation. The seaward migrations of smolts (and pink and chum fry) on the other hand are directed by active swimming in the pink, chum, and sockeye salmon, and by passive displacement coinciding with decreased swimming ability in Atlantic and coho salmon (Thorpe *et al.*, 1981; Smith, 1982). Smith (1982), using Raymond's (1968, 1979) data, argued that chinook smolts must also have a passive seaward displacement, and yet the basis for this is not supported by the U_{crit} data obtained in this study.

Thorpe and associates (1981) proposed that sockeye, essentially a lacustrine species, may have faced greater selection pressure for active directed migrations, than the more riverine species such as the Atlantic and coho salmon, which by necessity must abandon stream/river position-holding behaviours to enable their seaward migration. It would follow therefore, that the more oceanic and estuarine species (pink, chum and chinook) would tend not to exhibit the strong agonistic behaviours characteristic of riverine species (*see* Taylor (1990c) for a comparison of stream- and ocean-type behaviours in chinook salmon, and Hoar (1958) for a description of the behaviours of the pink, chum, coho and sockeye salmon), and hence active, directed swimming to the brackish and marine water would predominate over passive 'meandering' drift. A comparison of downstream behaviour of wild, stream- and ocean-type chinook with regard to active and directed, or passive and undirected swimming would be worthy of investigation. Field observations of the patterns of wild, migrant underyearling and yearling chinook smolts in this country, or movement-rhythm studies similar to those of Thorpe and associates (1988) would likewise be relevant with regard to elucidating whether the displacement is active or passive.

PARR-SMOLT TRANSFORMATION AND SEAWATER SURVIVAL

The ability of underyearling hatchery chinook salmon to survive a transfer to seawater was limited to a 'time window' (Boeuf and Harache, 1982) corresponding to the months of October, November, and December (late spring, early summer). Seawater survival of underyearling, wild smolts (approximately three month old, post-hatching fish) was absolute in the one seawater challenge test that was performed on such fish. Wild, yearling smolts (approximately 15 months post-hatching) did not survive beyond 96 hours in seawater in the laboratory however. The survival of hatchery fish therefore was coincident with the natural period of parr-smolt

transformation for this species (Unwin, 1986; Taylor 1990c; Healey, 1991). Seawater survival was strongly correlated with increased hypo-osmoregulatory ability as measured by gill Na^+-K^+-ATPase activity, salt-water induced losses of fork length and body weight, stability of blood haematocrit and the plasma variables of osmolality, and sodium and chloride ion concentration, and water content of the white muscle and viscera (Figures 5.1 to 5.5).

All the fish transferred to seawater with an enzymatic activity less than approximately 5 μmol P_i.mg protein^{-1}.h^{-1} died from osmoregulatory imbalance and tissue dehydration. In addition, comparison of gill Na^+-K^+-ATPase activity between 'initial' (freshwater), and '48 hour' (transferred to seawater) groups indicated that the chinook able to survive in seawater had the physiological capability to increase their enzyme activity some 25-40% upon direct entry to seawater. In the seawater transfer trials that caused fish mortalities, enzyme activity was not different between the groups (Figure 5.2). The rapid increase of enzyme activity upon seawater entry is in accord with earlier work carried out on chinook (Beckman and Zaugg, 1990).

The ability of underyearling 'smolts' to survive transfer to seawater as demonstrated in this study is in full agreement with the generalised natural history of ocean-type chinook salmon (Gilbert, 1913; Rich, 1920; Healey, 1980a,b, 1982, 1983; Taylor 1990a,b,c), and the stock from which they were derived (Clark, 1929; Kjelson *et al.*, 1982; Unwin, 1986; Franklin, 1989). Taylor (1990c) has recently demonstrated that both ocean- and stream-type chinook are capable of surviving a seawater challenge test at three months post-hatching. He also related that stream-type chinook had "*strikingly*" higher agonistic behaviour scores than the ocean-type population, and that these behaviours had a genetic basis and were related to the longer freshwater residence of stream-type chinook.

OUTLOOK FOR FURTHER WORK

At the risk of appearing political in the new 'user-pays' era, I feel that the Ministry of Agriculture and Fisheries' (MAF, although now entitled the National Institute of Water and Atmosphere, or NIWA) Glenariffe and Silverstream Hatcheries offer excellent facilities for the continued examination of alevin-smolt growth and development. Despite the current situation, with offshore trawlers harvesting large quantities of chinook, the ability to raise young fish for release under differing rearing regimes should be investigated further. The identical 'farm-raceways' situated at Glenariffe and Silverstream would permit the large scale rearing of chinook at differing flow rates (*i.e.* large scale exercise training studies). The effect of periodic sprint training periods on growth rates, and the various parameters of growth could also be readily assessed. Salt (sodium chloride) loading of pelleted salmon diets has not reduced growth rates of young salmonids previously (Shaw *et al.*, 1975a; Zaugg *et al.*, 1983). Furthermore, supplementary dietary salt has increased hypo-osmoregulatory ability of chinook salmon resident in freshwater (prior to their release), and successfully increased the recruitment rate to the fishery (Zaugg *et al.*, 1983).

All of these experimental treatments could be investigated (provided that the facilities and research funding were made available), and the effects of the manipulations related to overall smolt-adult survival. Closer co-operation, within New Zealand, between MAF (NIWA) and the university research groups (oft aired in conversation, but more often than not lacking in execution) through collaborative research and project proposals may increase the likelihood of securing the contestable 'research dollar', as both parties would be able to offer facilities and expertise. 'Co-incidentally' such scientific collusion would likely produce worthwhile research results for use by domestic aquaculture enterprise, and for comparison with studies abroad.

This study, like that of Franklin (1989) has determined that gill Na^+-K^+-ATPase is strongly correlated with survival and growth of chinook in seawater. Another physiological indicator of parr-smolt transformation and 'full smolt condition' is gill mitochondrial succinic dehydrogenase activity (SDH, Langdon *et al.*, 1984; Langdon and Thorpe, 1985). Physiological evaluation of SDH was not determined in this study. The assay protocol requires that the samples must be processed whilst fresh. The logistics of this study prevented this, however, a slightly different experimental design, or 'research group' type experiments would permit ontogenetic analyses of SDH activity in the New Zealand chinook before, during and after the period of parr-smolt transformation, as has been carried out for the Atlantic salmon (Langdon and Thorpe, 1985).

The practicality of determining seawater survival using a simple, non-invasive, non-physiological seawater challenge test was described in Chapter 5. The criteria for assessing seawater survival were salt water induced losses of fork length and body weight. The ease of this method readily lends itself to the commercial sector and follow up work should determine whether such a test (either a 24 or 48 hour) could be used to determine the 'seawater readiness' of yearling chinook smolts. Furthermore, a long term investigation could be carried out by MAF (NIWA) or some other ocean ranching concern to determine whether adult return of ocean ranched fish is correlated to seawater survival and osmoregulatory performance during a 24 or 48 hour test. The importance of separate environmental parameters on the timing of the parr-smolt transformation and associated seawater survival could be individually investigated with controlled experimentation, relative to standard 'data base' data regarding the changes associated with a standard 24 or 48 hour seawater challenge test.

The 'poor' swimming ability of New Zealand sockeye provokes questions as to the swimming ability of the voluntarily landlocked chinook populations found within several freshwater habitats in this country. Studies comparing and contrasting the swimming ability of such fish with those from sea-run stocks would be propitious in the light of the research of Taylor and Foote (1991) on pure bred stocks of sockeye and kokanee.

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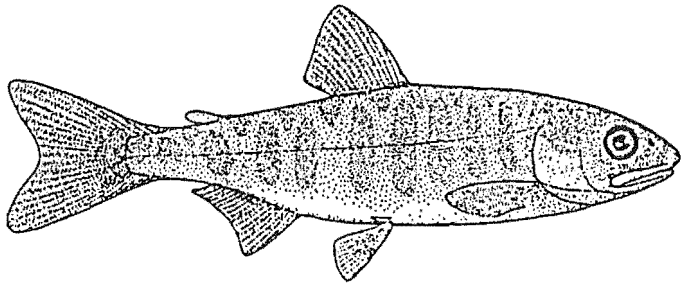

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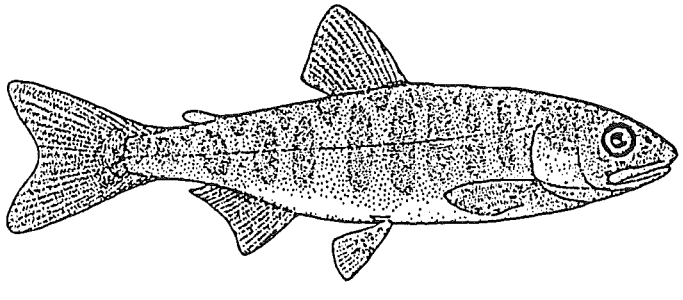

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